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H. W. WILEY, Chief of Bureau.

STUDIES ON APPLES.

- I. STORAGE, RESPIRATION, AND GROWTH.
II. INSOLUBLE CARBOHYDRATES OR MARG.
III. MICROSCOPIC AND MACROSCOPIC EXAMINATIONS OF APPLE STARCH.

BY

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LETTER OF TRANSMITTAL

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF CHEMISTRY,

Washington D. C., April 12, 1905.

SIR: I have the honor to transmit herewith the manuscript of a report on some studies made in the division of foods and in the microchemical laboratory of this Bureau on the development of apples, especially under different conditions of storage, and to recommend the publication of the same as Bulletin No. 94 of the Bureau of Chemistry.

This investigation has been in progress for the past two years, and some preliminary work was done prior to that time by Mr. J. S. Burd. The work was originated, planned, and directed by Mr. W. D. Bigelow, chief of the division of foods. The analytical and bibliographical data should be, for the most part, accredited to Mr. H. C. Gore, though Mr. E. M. Chace and Mr. W. L. Dubois participated in the analytical work. The third section of the report, comprising the microscopic studies, photographs of the fruit, and the specific gravity determinations, is entirely the work of Mr. J. B. Howard, chief of the microchemical laboratory.

The investigation has been made in collaboration with Messrs. William A. Taylor and G. Harold Powell, of the office of pomological investigations, Bureau of Plant Industry, who have selected and procured the samples and furnished facilities for their transportation and cold storage. They have also taken an active interest in planning the work and discussing its results, and have furnished all of the pomological data.

Very respectfully,

H. W. WILEY, *Chief.*

Hon. JAMES WILSON,
Secretary of Agriculture.

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STUDIES ON APPLES.

I. STORAGE, RESPIRATION, AND GROWTH.

REVIEW OF WORK ON THE RIPENING AND RESPIRATION OF FRUITS.

In the following résumé of works on this subject, the review of Gerber^a has been used as a guide to the French memoirs on the subject.

The earliest source available in the original was the paper of Bérard.^b This author carried on elaborate researches on the ripening and respiration of fruits, his results apparently contradicting those of De Saussure,^c whose conclusions were considered not to be sufficiently well established by experiments, De Saussure having put forth the idea that green fruits act like leaves toward the atmosphere surrounding them, in light or darkness.

Bérard studied the effect produced by unripe strawberries and many other unripe fruits on the air surrounding them by keeping them for twenty-four-hour periods in air in large flasks sealed by mercury. He found in all cases an increase of carbon dioxid at the expense of oxygen, and in no case the reverse change. Similar experiments were tried on fruits still attached to the tree (apricots, peaches, and plums), with the result that the fruit did not mature, but became withered and browned, showing apparently that oxygen is necessary for the ripening of fruits. Experiments are also described in which fruits were kept for long periods of time in inert gases—carbon dioxid, hydrogen, or nitrogen, and in vacuo. These experiments were not considered successful, for it was found that the fruit lost its odor and acquired a disagreeable taste.

In the second contribution^d methods for the analysis of fruits are presented, and analyses given of many fruits when green and when ripe, viz, apricots, currants, cherries, plums, peaches, and pears.

De Saussure^e confirmed his previous work, published in 1804, by a report of new experiments, which differ widely in their results from

^a *Annales des sciences naturelles*, 1896 (8), 4: 1.

^b *Ann. chim. phys.*, 1821 (2), 16: 152, 225.

^c *Recherches chimiques sur la végétation*, Paris, 1804.

^d *Ibid.*, p. 225.

^e *Ann. chim. phys.*, 1821 (2), 19: 143, 225.

those of Bérard.^a The air in which the fruit was kept was examined at shorter intervals than in the experiments of Bérard, and this may account for the disagreement of their results, though De Saussure believed that the glass containers used by Bérard were too small, so that crowding or overheating may have taken place.

De Saussure reiterated that green (unripe) fruits act on the air like leaves, differing only in intensity of action, which is less in the case of fruits. During the night oxygen disappeared and was replaced by carbon dioxide, which was partly absorbed by the fruit, absorption being less in free than in confined air. The fruits consumed, volume for volume, more oxygen in the dark when they were very green than when they approached maturity. On exposure to sunlight they gave off part or all of the oxygen of the carbon dioxide which they had absorbed in darkness, and used up all the carbon dioxide from the atmosphere in which they were inclosed. Green fruits could even remove the carbon dioxide from an atmosphere artificially charged with several per cent of the gas.

Couverchel^b contributed two papers of much interest, in which the ideas of several early writers on the subject are given. These ideas were founded largely on theory rather than on experiment. From the early work of Sennebier are quoted the following items of interest:

The fruits which have yellowed in ripening are more succulent than green fruits, are nearer decay, are more gummy than resinous, and are more soluble in water. Perhaps the phlogiston may have less energy because it is more attenuated, the fiber incenses, the mass of the fruit increases, etc.

Sennebier supposed that the fruit suffered a loss in phlogiston (corresponding to a gain in oxygen). In a later work he says: "The taste of fruits, at first bitter, becomes acid, then sweet. The astringent principle which appears before the formation of vegetable acid changes to sugar by oxidizing." This writer considered that gallic acid was the "unfinished" vegetable acid, completed by the oxygen which it appropriated. "It is certain," he adds, "that the acids oxidize more and more; for example, citric acid in green grapes passes, by oxidation, into tartaric acid." This idea appears to have been current among the chemists of the time, since it was specifically denied by Frémy in 1844 in the case of grapes. (See Frémy, p. 11.)

Other early writers are quoted by Couverchel, showing at what an early date the functions and fate of the changing constituents of growing fruits were studied and how varied were the notions concerning them. For example, Lamarek and Decandolle considered that oxygen arising from the decomposition of carbon dioxide acted on the mucilage of the fruit, changing it to sugar. Berthollet thought

^a Loc. cit.

^b J. pharm. chim., 1821 (2), 7:249; and Ann. chim. phys., 1831 (2), 46:147.

that the acid flavor of green fruits was produced by oxygen, which was only very feebly held in combination in the fruit.

This first paper by Couverchel gives results of the analysis of juice of apricots and grapes at different stages of growth, determinations of density, acidity, dry weight (in vacuo), gum, sugars (weighed as dried sirup), ash, and soluble ash.

In the second contribution, in 1831,^a further quotations from early writings are given. Of much interest is that from the work of Ingenhouz:^b

All fruits, day and night, exhale a mephitic air, and possess the power to render the surrounding air unwholesome. I have been very much astonished to find a poison in fruits which are so much eaten, the more since the finest fruits possess this power in high degree. * * *

Couverchel differed from his predecessors, believing the presence of air to be only incidental in the ripening of fruits, and that ripening goes on by action of principles contained in the fruit. It may be, he says, that the sap becomes acidified in its passage through the young branches to the ovary by reason of the decomposition of water and absorption of the oxygen. The acids so formed may act on the gelatin and give rise to sugars, sugars being considered as intermediate substances between the mucilages and the vegetable acids, containing more oxygen than the former and less than the latter.

In 1844 Frémy^c discussed the work of Bérard on the respiration of fruits. He confirmed the observation that fruits consume oxygen, giving off carbon dioxid, and carried out an experiment similar to those of Bérard. In Frémy's work the unripe fruit, attached to the tree instead of being kept in a closed jar as in the experiments of Bérard, was coated with varnish, in this way stopping the normal respiratory changes. Growth was found to cease, as in the experiments of Bérard.

The air contained in green and in ripe fruits was analyzed by boiling slices of the fruit in brine and analyzing the air which separated. The presence of a ferment causing the respiratory changes was suggested, although a pear, after it was ground, gave off no carbon dioxid gas, whereas before grinding carbon dioxid was freely evolved.

The old idea that the acids change in ripening (see p. 10) was refuted in the case of grapes, by the recognition of tartaric acid in the very young fruit.

Unripe fruit was profoundly altered by soaking in dilute sodium carbonate, but no conclusions are drawn. Objection is made to the statement that starch can form the sugar found in fruit, since the sugar

^aLoc. cit.

^bVersuche mit Pflanzen, 1786, 1: 64; 2: 61, 221, through Gerber, loc. cit.

^cCompt. rend., 1844, 19: 784.

(glucose) formed from starch by the action of acids was found by Biot to be not the same as the sugar in fruits.

Some of the ideas of Frémy were vigorously combated by Couverchel.^a

A very valuable contribution to the subject of the analysis of fruits by Buignet,^b which appeared in 1860, is of interest because of the methods given for the determination of many constituents of the strawberry. Water, acid, sugars, fat, soluble and insoluble protein, marc, nitrogen-free parenchyma, pectin, odoriferous principles, coloring matters, and ash are worked out. Strawberries containing the least water are said to contain the most sucrose, and this is explained on the hypothesis that the acid and sucrose are contained in different cells, and in the presence of much water these diffuse more readily than in drier berries, a more rapid inversion ensuing. Sucrose is believed to be the initial sugar formed. Results for many varieties of strawberries are tabulated. A later paper^c gives results concerning sugars in fruit in the form of a summary of 14 conclusions, some of which are as follows: Sucrose is found in many fruits, becoming inverted in the ripening, sometimes completely, e. g., in grapes, currants, and figs; sometimes partially, e. g., in bananas, apricots, peaches, plums, apples, and pears. No relation exists between sucrose and the acidity of fruits, but the sugar is probably inverted by a nitrogenous ferment. This was proved by showing that, after precipitating with alcohol, the sucrose remained unchanged; that after neutralizing an original sample of juice with calcium carbonate, the inversion still went on; and that in bananas, which have no free acid, starch is rapidly changed to sucrose. Invert sugar prevents the crystallization of sucrose, but the latter sugar was isolated in crystals from a number of fruits by a procedure which is described. Starch was not found in fruits (except the banana), but a tannin-like, astringent principle is described as occurring in unripe fruits, which decolors an iodine solution, yielding a precipitate which, when treated with acid, yields a glucose sugar, the same as that formed from nut galls. The starch and tannin found in the banana are said to disappear simultaneously, giving rise to cane sugar. Finally we are told that a difference exists between sugars of fruits according as they are produced under action of vegetable forces, or without it, e. g., bananas contain more sucrose and less invert sugar when allowed to ripen on the tree than if ripened after picking.

Berthelot and Buignet^d worked on the ripening of oranges. Two samples of green oranges were studied. Several fruits from each

^a Compt. rend., 1844, **19**: 1114.

^b J. pharm. chim., 1859 (3), **36**: 81-111 and 170-198; résumé in Compt. rend., 1859, **49**: 276-278.

^c Compt. rend., 1860, **51**: 894.

^d Ibid.: 1094.

sample were analyzed, the remainder being kept for some time in a dry place at an even temperature, when another analysis was made. No figures were given, and changes in the sugar only are reported, but these changes were remarkable, because, while the invert sugar remained nearly constant, the sucrose increased, calculated either on the basis of juice or on that of soluble solids. This was considered as a very curious change to take place in an acid fruit. It is the more notable when it is remembered that the green fruit contains no starch.

Cahours^a noted that ripe oranges evolved carbon dioxid, consuming oxygen at the same time, and that when all the oxygen was used up the carbon dioxid evolution still continued. It also continued in an atmosphere of nitrogen.

Chatin^b considered that the carbon dioxid evolved by ripening fruits resulted from the oxidation of the tannin, since, as the fruit ripened, tannin disappeared.

Frémy^c called attention to the three periods in the life history of a fruit—growth, ripening, and decay.

Corewinder^d gave an analysis of ripe bananas, and later^e studied bananas from ripeness to decay during a period of eighteen days, during which sugars were determined at 10 different times. The sucrose fell from 15.90 to 2.84 per cent; total sugar from 21.80 to 14.68 per cent; and invert sugar increased from 3.90 to 11.84 per cent.

Beyer^f studied the growth of gooseberries, analyzing them every few days from the time when the fruit was very small until it was ripe. The complete analysis of the fruit at each picking is reported, including sugar, acid, protein, ash, fat, and nitrogen-free residue. No determinations of sucrose were made.

A notice of work done by Pasteur in 1866 is found in a review of literature on the ripening of grapes by Fitz (p. 14). Pasteur cast doubt upon the idea that the acid in ripening grapes gave rise to sugar, by discovering that in sour varieties the acid actually increased during ripening.

Petit^g published discussions on the ripening of grapes. He considered that during ripening the sugar came from the cellulose in the fruit, the cellulose first changing to acid by oxidation, and the acid then becoming sugar with the evolution of carbon dioxid. Both sucrose and reducing sugar were found in the leaves of the grape, cherry, and peach, but in the grape itself only reducing sugar was present.

^a Compt. rend., 1864, **58**: 495 and 653.

^b Ibid., 1864, **58**: 576.

^c Ibid., p. 656.

^d Ibid., 1863, **57**: 781.

^e Ann. agron., 1876, **2**: 429.

^f Landw. Versuchs-Stat., 1865, **7**: 355.

^g Compt. rend., 1869, **69**: 760; and *ibid.*, 1873, **77**: 941.

Dupré,^a however, noted a positive increase in acid in grapes during ripening, disproving the conclusion of Petit.

Neubauer^b studied the ripening of grapes. The complete analysis of two varieties of grapes at successive stages of growth is presented. No satisfactory explanation could be given for the great increase of sugar that took place on ripening.

Famintzin^c published the results of a study of the ripening of grapes, which is of much interest because it explains the hitherto unknown source of the sugars which appear in the grape on ripening. He used both chemical and microscopical methods. Twelve analyses of a variety of growing grapes are given and the results discussed. The unripe grape contains no starch, but the stems of the fruit are full of starch, which disappears as the grape ripens. Tannin was present throughout the very young fruit, but later occurs only in the outer layer of the fruit pulp and in the embryo.

This work was confirmed by Hilger,^d who followed the changes of water, ash, sugar, and acid content in the leaves and fruit of the grape during growth. The stems of the fruit were found to be full of starch, which disappeared when the rapid increase of sugar began. The increase of sugar for a period of six days at the ripening time was from 3.87 to 7.70 per cent in the case of one variety, and from 5.33 to 7.71 per cent in another, during which time the starch in the stems disappeared.

Mercadante^e believed that the sugars were formed at the expense of the gummy matters in the fruit, and also from acids, the fruit being thus considered to be the seat of the formation of sugar. Macagno^f showed that sugar formation takes place in the leaves, not in the fruit of the grape.

Saintpierre and Magnien^g held a similar view. These authors first give a review of the literature on fruit ripening. They found that ripening fruits give off carbon dioxide in light or darkness, that they absorb or give off water according as they are kept in a moist or dry place, and that acid and sugar come to the fruit through the stem. The acid is believed to be used up in respiration, the sugar meanwhile concentrating in the fruit until in its turn it furnishes the carbon dioxide for combustion.

Pollacci,^h studying the after ripening of grapes, found that the sugar increased and the acid decreased in grapes stored after picking.

^a Weinlaube, 1870, p. 274, through A. Fitz, *Ann. Oenol.* **2**: 241.

^b Landw. Versuchs-Stat., 1869, **11**: 416.

^c *Ann. Oenol.*, 1871, **2**: 242.

^d Landw. Versuchs-Stat., 1874, **17**: 245.

^e *Gazzetta chimica Italiana*, 1875, 5th ser., p. 125, through Gerber, loc. cit.

^f *Compt. rend.*, 1877, **85**: 763, 810, through Gerber, loc. cit.

^g *Ann. agron.*, 1878, **4**: 161.

^h *Ibid.*, 1877, **3**: 629.

Lechartier and Bellamy^a published two papers which give results of experiments with apples stored in sealed vessels. Large quantities of carbon dioxid gas were evolved during the long periods of time in which the fruit was under observation. Very considerable quantities of alcohol were found, and the presence of yeast cells in the parenchyma cells of the sound apple is noted. These observations have never been confirmed, neither alcohol nor yeast cells occurring in an apple whose skin is intact; however, so far as is known no work has been done since on apples held in sealed chambers.

Pfeiffer^b gives a review of the chemical study of fruits. In the same paper are given the results and a discussion of the study of the growth of apples and pears. Crude fiber, ash, protein, sugar, acid, water, and pectin and dextrin, are all shown to increase during growth, the constituents of the apple increasing more rapidly in the three varieties studied than those of the pear, of which two varieties were used.

Mach^c carried on work on the growth and ripening of grapes and later^d extended the work to other fruits, viz, apples, pears, mulberries, strawberries, red and black currants, cherries, and peaches. With grapes he found that the fruit grows rapidly in size until it begins to color, then grows more slowly. Sugar develops slowly at first, but after the coloring of the grape, very rapidly. The percentage of acid remains about constant till ripening begins, when it falls. Tannin is present in the largest quantity at first. Starch is present in the growing shoots in the leaves and in the grape stems. It disappears, however, as the fruit ripens. Unfortunately, with the other fruits studied, no determinations of sucrose are made.

Ricciardi^e followed the ripening of bananas, the starch being found to give rise to sucrose. The author agreed with Buignet^f that in the banana ripened on the tree there is no invert sugar, but this point is not brought out in the analysis given. No alcohol was found in the over-ripe fruit.

The work of De Luca is used as a basis for further work by Gerber^g on the ripening of olives. In De Luca's paper^h a table showing the growth of the olive from June to February is given. Thirty-four examinations were made on different dates to determine the average weight and density. Picked olives yielded more oil when they were allowed to stand in oxygen, or in air in daylight, than when analyzed at once or after they were kept in carbon dioxid. The presence of a bitter principle was noted in green olives, removable by prolonged soaking in water. Mannite was found in considerable quantity in the fruit and leaves of the olive tree and was isolated by extracting with

^a Compt. rend., 1869, **69**: 356, 466.

^b Ann. Oenol., 1876, **5**: 271.

^c Ibid., 1877, **6**: 409.

^d Ibid., 1879, **8**: 46.

^e Compt. rend., 1882, **95**: 393.

^f Loc. cit.

^g Compt. rend., 1897, **125**: 658.

^h Ibid., 1861, **53**: 380.

hot alcohol. It was believed by the author to be essential for the production of oil, though this could not be proved.

In a second contribution^a the results of successive analyses of the pulp of the olives for oil are given. The percentage increased by large increments, but irregularly (probably because of the small sample used—three fruits) with the growth. The presence of a chlorophyll-like body in the pulp was observed, becoming less in quantity as the fruit approached ripeness. The proportion of pulp to pit is given.

The third paper^b discusses mannite. Determinations of mannite in the fruit during growth are given. It was found to occur in widely varying amounts up to 1.54 per cent of the dry matter. The mannite and the chlorophyll-like body disappeared as the fruit ripened and seemed to be closely connected with the formation of oil.

Upon the results of the above work, and from his own experiments on the respiration of olives, Gerber^c considers that he has established direct proof of the transformation of sugar-like bodies, especially mannite, into oils. When the respiratory quotient $\frac{\text{CO}_2}{\text{O}_2}$ (ratio by volume of carbon dioxid produced to oxygen consumed) is greater than unity, and no acids are disappearing to furnish the extra carbon dioxid by breaking down (which he believes takes place in acid fruits), mannite is believed to be passing over into olive oil with the evolution of water and carbon dioxid.

Roussille^d presented consecutive analyses of the leaves and fruit of the olive tree during the growing season. He determined that the oil did not undergo migration from the leaves to the fruit, but was formed in the fruit.

Funaro^e gives a short review of previous work, noting that of De Luca (see p. 15) and the paper of Harz,^f and concludes that the oil is formed by special secreting cells containing an unknown material. The author then gives his results in tabular form, which represent the growth of the olive, the weight and dimensions of the fruit, the moisture content of flesh and stone, and the total ether extract in flesh and stone.

Mannite was found in small quantities in fruits and leaves. Its presence in fruits at the end of the growing season contradicted the conclusions of De Luca, who states that it disappears as the fruit increases in oil. According to Funaro this indicates that mannite probably has nothing to do with the formation of oil.

This view is in accordance with conclusions reached by Hartwich and Uhlmann.^g These authors considered glucose to be the material

^a Compt. rend., 1862, **55**: 470.

^b Ibid., **55**: 506.

^c Ibid., **125**: 658.

^d Ann. agron., 1878, **4**: 230.

^e Landw. Versuchs-Stat., 1880, **25**: 52.

^f Ann. prak. Pharm., **19**: 161.

^g Arch. Pharm., 1902, **240**: 471.

from which oil is formed, and affirm that mannite does not occur in the fruit of the olive.

Keim^a studied changes in the composition of the flesh of cherries at intervals of from seven to ten days. As the fruit ripened the percentage of water decreased, and both acid and sugar increased with total dry matter. In the early stages of growth, citric, malic, and succinic acids were present, but nine days before ripeness the succinic acid disappeared. Dextrose and levulose are always present, while inosite, which at first was present in appreciable quantity, diminished to a mere trace. Sucrose was present in small amount. No starch was found in the fruit save in the outer green layer of the very young fruit, but it occurred in the parenchyma cells of the fruit stem in increasing amounts as the fruit ripened.

Kulisch^b presented a valuable discussion of the changes in the constituents of ripening fruits, giving also an account of a study of the changes which a variety of apples underwent on storing at cellar temperature. The fruit from two trees of the same variety in the same orchard were employed and analyzed separately, so that the results have further interest in showing how the composition of fruit from different trees of the same variety, grown under the same cultural conditions, may vary. (See p. 31 and fig. 6, p. 30.)

The growth of a single variety of apples was thoroughly studied by Lindet,^c who examined the fruit at fifteen-day intervals through the growing season (see p. 24 and fig. 29, p. 63). He established the fact that the acid content gradually became less, and that the starch increased by degrees until the fruit began to ripen, when it decreased gradually. Sucrose and invert sugar increased steadily up to the last analysis. A portion of each sample received was left in darkness and analyzed at intervals. The starch decreased gradually to about 0.8 per cent, sucrose and invert sugar increased, while total carbohydrates fell, allowance being made for loss of moisture by the fruit on being kept. These changes are recognized as taking place: (1) The change of starch into sucrose; (2) the inversion of sucrose; and (3) the consumption of invert sugar in respiration. The change of starch into sucrose is said to be a chemical phenomenon whose mechanism escapes us. The localization and disappearance of starch in apples is described, and a description of the starch grains is given.

C. A. Browne, jr.,^d noted the starch and sugar changes of a sample of Baldwin apples, picked green, obtaining results similar to those of Lindet and those obtained in the Bureau of Chemistry. Otto^e also

^a Zts. anal. Chem., 1892, **30**: 401, abstr. in Agr. Science, 1892, **6**: 387.

^b Landw. Jahrb., 1892, **21**: 871.

^c Ann. agron., 1894, **20**: 5-20.

^d Penn. State Dept. Agr., Bul. 58.

^e Centrbl. agrikulturchem. (Biedermann), 1902, **31**: 107.

studied the growth and ripening of a variety of apples, noting that the percentage of water, acid, and starch decreased more or less uniformly, while dry matter, extract, sucrose, and reducing sugar increased. In another contribution by Otto^a a study of the changes which go on in common storage is presented. Samples of eight varieties of apples were analyzed before and after cellar storage. A gradual diminution of all constituents was found except in two cases in which, while the acid and starch decreased, the total sugar showed a slight increase.

In further work done by Otto^b it was found that the starch in ripe apples when they were allowed to sweat in piles was entirely converted into sugar in two or three weeks, the fruit thus becoming more valuable for cider making.

The contribution of Gerber^c to the subject of the ripening of fruits is of considerable importance. The work is of some length, occupying 280 pages. First, the work of various writers on respiration and carbohydrate, acid, and tannin changes in growing and ripening fruits is reviewed, together with the resulting hypotheses regarding these functions. The author then describes the methods and apparatus employed by him in the researches which are described. The relative intensity of respiration and the value of the respiratory quotient, $\frac{\text{CO}_2}{\text{O}_2}$, are the chief criteria used. The fruit (a single fruit in case of apples) is held in a sealed glass container, and the air of the chamber is analyzed after a time. The air is then renewed and the experiment repeated, the temperature being held constant. In this way the intensity of respiration and the respiratory quotient—i. e., the ratio of carbon dioxide given off by the fruit to the oxygen consumed—may be observed.

The method is first employed with apples. It is noted that unripe apples breathe much more rapidly than ripe apples. The respiratory quotient, $\frac{\text{CO}_2}{\text{O}_2}$, is found to be greater than unity in the case of growing apples, but it is considered that it is impossible to determine to what constituent this fact is due, because, although the constituent, malic acid which is suspected of furnishing the extra carbon dioxide, decreases relatively to the other constituents (though increasing in absolute quantity) during growth, it is impossible to determine whether or not the sap which is supplied to the apple varies in composition during the period of growth. More definite results are considered to be obtained with apples during the period of after-ripening—i. e., ripening after picking—because here no sap is added to the fruit. Apples

^aCentrbl. agrikulturchem. (Biedermann), 1902, **31**: p. 104.

^bLandw. Versuchs-Stat., 1902, **56**: 427.

^cLoc. cit. (see p. 9).

were examined during this period. The influence of temperature, quantity of acid present in the fruit, variety of apple, and the effect of quartering the apples on the intensity of respiration and on the respiratory quotient are described. Apples respire more rapidly at 30° and at 33° than at 18° C. Apples relatively high in acid respire more rapidly and have a higher respiratory quotient than apples low in acid. The influence of cutting the apple into quarters is to increase the intensity of respiration and respiratory quotient.

Similar researches were carried out with grapes, citrus fruits, almonds, peaches, plums, and apricots. The existence of a respiratory quotient greater than unity due to the combustion of acids is considered by the author to be established, and numerous conclusions concerning the effect of various factors on the function are given.

Work with molds is described, the respiratory quotient of these organisms growing on different media being determined, and the results are believed to furnish direct support to the conclusions which the author has drawn from his work with fruits.

Other fruits, Japanese persimmons (*Diospyrus kaki zendji*), bananas, sorbes (*Sorbus domestica*), and medlars (*Mespilus germanica*) were now examined by the method of the author. In persimmons tannin is believed to disappear by direct combustion, with no formation of sugar, this conclusion, however, depending on the results of an analysis of a single persimmon.

The conclusion reached with the acid fruits, that the combustion of the acids is reponsible for the excess of carbon dioxid given off over oxygen consumed, possesses a certain probability merely because of the uniformity of the results obtained with a wide range of varieties of acid fruit. Gerber's work is valuable as suggesting certain auxiliary work that may be carried on in connection with the chemical work necessary to the study of the ripening of fruit. His theories should be considered as hypotheses which must be confirmed by suitable scientific methods before they can be seriously regarded. Such data should be considered as indications of facts only. The number of individual specimens in the samples examined and the methods employed do not warrant that any greater weight be given to the data. At the same time it is shown that the examination of the respiratory changes in fruits is easily made, and that fruits of different varieties and different degrees of maturity vary materially in the intensity of their respiration. The method would probably be of value in supplementing chemical work on growing or ripening fruits.

From the above review it will be recognized how varied are the conclusions reached by workers with ripening fruits. With better chemical knowledge, particularly concerning sugars and starch, satisfactory agreements are found between the work of various modern authors working with apples and grapes. It must be considered that

each variety of fruit presents a different set of relations between its constituents, and that each fruit contains a variety of undetermined substances in considerable amount which make changes between known constituents difficult to demonstrate. No general conclusions can therefore be reached until each kind of fruit is worked with as far as possible through the stages of growth and ripening, and only as the undetermined material in fruits is gradually worked out will the chemistry of fruits become clear.

THE RIPENING OF APPLES IN COMMON STORAGE.

In the summer of 1901 the preliminary work on the ripening of fruit was begun. Eight varieties of apples were selected, and each one was examined several times, the chief object being the study of methods of analysis. No consecutive data were obtained, but the experience gained was valuable in beginning the work in the following year. This preliminary work on the subject was done principally by Mr. J. S. Burd.

DESCRIPTION OF THE VARIETIES ANALYZED.

The following descriptions of the varieties of apples employed in the investigation is furnished by the pomologist of the Bureau of Plant Industry:

BEN DAVIS.

Fruit medium to large, yellowish, splashed and striped with red. Flesh somewhat tough and lacking in juice; flavor mild subacid. A winter variety of excellent keeping quality.

BOUGH (synonym *Sweet Bough*).

Fruit above medium in size, pale greenish yellow. Flesh very tender, with a rich, sweet flavor. An early summer variety ripening over a period of two or three weeks. Keeps but a few days under ordinary conditions after being picked from the tree.

EARLY STRAWBERRY.

A small roundish apple striped and stained with bright and dark red. Flesh tender, with a sprightly, brisk, subacid flavor. A midsummer variety in season for several weeks.

HUNTSMAN (synonym *Huntsman Favorite*).

Fruit large, pale yellow, sometimes shaded with pale red or deep yellow in the sun. Flesh rather coarse, tender, of a mild, rich, subacid flavor. A winter variety of fairly good keeping quality.

NORTHERN SPY.

Fruit large, greenish yellow, covered with light and dark stripes of purplish red. Flesh tender, with a fine, sprightly subacid flavor. A winter variety of good keeping quality under favorable conditions, but quite subject to decay, if roughly handled, on account of thin skin and tender flesh.

RHODE ISLAND (synonym *Rhode Island Greening*).

Fruit medium to large, dark green, becoming greenish yellow when ripe. Flesh tender, crisp, with a rich, acid flavor. An early winter variety of fair keeping quality, quite subject to scald after midwinter, except under very favorable conditions.

WINESAP.

Fruit medium in size, of a fine dark red color. Flesh firm, crisp, with a rich, subacid flavor. A winter variety of excellent keeping quality, except for being somewhat subject to scald in late winter and spring.

WINTER PARADISE.

Fruit rather large, dull green, with a brownish blush or faint striping. Flesh fine-grained, sweet, and sprightly, with a distinctive and marked aroma. A winter apple of good keeping quality.

YELLOW TRANSPARENT.

Fruit medium in size, clear greenish yellow in color. - Flesh very tender; flavor sprightly, quite acid; one of the earliest of the summer apples. Ripens over a brief season and keeps but a short time.

DESCRIPTION AND ANALYSES OF SAMPLES.

In the summer of 1902 the study was again taken up, four varieties being selected from three localities. The description of the samples of apples studied and such cultural data as were obtained are given in Table I. The analyses of the apples made from time to time while the samples were held in common storage are given in Table II. These results were also calculated to the basis of total solids and platted on graphic charts (figs. 1-4 and fig. 8), the actual changes in composition during the growth and development of the fruit being illustrated much more clearly in this way than by means of the numerical tables of analyses. In all of these charts the ordinates give the percentage of the various constituents and the abscissæ the dates on which the examinations were made.

TABLE I.—*Descriptive and cultural data for samples of apples studied—1902 and 1903.*

No. of first analysis	Date of first analysis	Stage of growth and condition	Variety	Grower	Locality	Cultural notes
1902						
1095	Aug. 25	Later stages of growth and common storage	Rhode Island Greening	G. G. Hitchings	South Onondaga, N. Y.	Soil, clay loam, stony, with heavy red clay or gravel, and clay subsoil; altitude about 1,200 feet; orchard sprayed; sod culture with grass left in orchard for mulch.
1096	Oct. 20	do	Northern Spy	do	do	do
1145	Oct. 20	do	Ben Davis	do	do	do
1123	Oct. 6	Later stages of growth and common storage	Winesap	S. H. Derby	Woodside, Del.	Soil, sandy, with clay loam subsoil; altitude about 60 feet; trees 10 to 25 years old; thorough spraying and tillage; annual use of clover cover crops; trees unusually vig- orous.
1144	Oct. 20	Common storage	do	do	Xenia, Ill.	No record of cultural conditions.
1146	Oct. 25	do	Ben Davis	do	do	do
1147	do	Cold storage	do	do	do	do
1148	do	do	do	do	do	do
1143	Oct. 20	do	Winesap	do	do	do
1142	do	do	do	do	do	do
1903						
6120	June 11	Growth	Early Strawberry	F. M. Soper	Magnolia, Del.	Soil, sandy, with clay loam subsoil; altitude about 60 feet; thorough spraying and tillage.
6122	do	do	Bough	do	do	do
6121	do	do	Yellow Transparent	do	do	do
6193	June 16	do	Ben Davis	G. P. Miller	Gerrardstown, W. Va.	Soil, soapstone derived from Romney shale, clay subsoil; altitude 700 feet; spraying and cultivation.
6494	do	do	Huntsman	do	do	do
6495	do	do	Winter Paradise	do	do	do

TABLE II.—Consecutive analyses of apples held in common storage.

Serial No.	Variety.	Date of analysis.	Acid as malic.	Starch. ^a	Polarization.		Sucrose.		Reducing sugars as invert.	Total Cellulose.	Pentosans.	Alcohol precipitate.	Nitrogen.		Total ash.	Alkalinity of ash. ^b	Specific gravity of whole fruits.
					Before inversion.	After inversion.	By polarization.	By reduction.					Total nitrogen.	Soluble nitrogen.			
			Per ct.	Per ct.	°V.	°C.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	Per ct.	
1095	Rhode Island Greening	1902, Aug. 25	13.67	2.49	3.0	—	5.39 at 25	1.82	4.58	76.91	1.01	1.01	0.052	0.025	0.38	0.22	0.8837
1120	do.	Sept. 15	14.92	2.26	3.6	—	6.6 at 19	2.26	5.09	7.64	1.15	1.14	0.045	0.028	0.659	0.24	0.8297
1127	do.	Oct. 6	15.14	1.83	2.9	—	7.2 at 20.8	2.63	5.39	8.78	1.09	1.02	0.036	0.018	0.86	0.21	
1155	do.	Nov. 7	15.68	1.70	2.7	—	9.0 at 20	4.76	4.66	6.29	1.16	0.96	0.036	0.015	0.824	0.21	
1177	do.	Dec. 1	15.50	1.64	2.7	—	8.7 at 21	3.79	3.68	7.03	1.16	0.96	0.036	0.015	0.862	0.21	
1260	do.	Dec. 19	13.56	1.46	3.6	—	9.25 at 20	2.76	2.74	8.08	10.97				0.35	0.21	0.8272
1694	do.	Dec. 31	13.84	1.46	3.9	—	9.1 at 20	2.42	2.08	10.97							
2021	do.	1903, Jan. 15	16.31	1.47	6.6	—	9.8 at 22.5	4.24	1.75	9.15	10.96				0.34	0.23	0.8083
2022	do.	Jan. 17	16.31	1.47	6.6	—	9.8 at 24	1.84	1.66	9.66	11.41				0.34		0.8083
2618	do.	Jan. 27	16.61	1.45	6.7	—	8.8 at 21	1.59	1.49	9.59	11.16				0.34		0.8083
2719	do.	Feb. 5	17.17	1.51	6.7	—	10.4 at 22	4.12	1.95	10.69	11.69				0.34		0.8083
2830	do.	Feb. 11	17.35	1.32	7.2	—	8.8 at 25	1.23	1.04	9.97	11.06						
1096	Northern Spy	1902, Aug. 25	14.60	2.61	3.85	—	6.05 at 25	1.67	1.94	5.06	7.10	0.80	0.032	0.007	0.285		
1121	do.	Sept. 15	15.39	2.58	4.5	—	7.59 at 19	4.23	2.19	5.80	8.10	0.86	0.025	0.010	0.069		
1128	do.	Oct. 6	16.03	1.71	3.6	—	7.80 at 20.8	4.23	3.02	6.03	9.21		0.028	0.010	0.329	0.188	0.8513
1154	do.	Nov. 7	16.22	1.70	3.6	—	9.3 at 20	4.91	4.80	6.90	12.06	0.80	0.025	0.007	0.028	0.188	0.8513
1178	do.	Dec. 1	15.70	1.56	3.6	—	10.3 at 21	3.78	3.80	7.87	11.86	0.74	0.030	0.013	0.059	0.269	0.8275
1261	do.	Dec. 19	15.63	1.42	5.6	—	9.1 at 20	2.64	4.23	8.88	411.30	0.32	0.030	0.013	0.298	0.269	0.8092
1695	do.	Dec. 31	15.76	1.34	7.15	—	9.9 at 20	2.08	4.77	9.49	411.41						
2020	do.	1903, Jan. 15	18.18	1.44	7.9	—	10.5 at 22.3	1.98	1.75	11.32	13.16				0.362		
1126	Winesap	1902, Oct. 6	15.60	1.51	4.10	—	7.7 at 20	1.87	2.02	7.68	9.81		0.033	0.008	0.397	0.264	0.8529
1156	do.	Nov. 7	16.92	1.37	3.0	—	7.8 at 20	3.63	3.55	8.23	11.78	0.73	0.025	0.007	0.282	0.195	
1169	do.	Nov. 25	16.02	1.50	4.8	—	8.2 at 20	4.27	2.21	9.74	12.07	0.73	0.025	0.007	0.017		
1181	do.	Dec. 9	17.04	1.42	6.5	—	9.15 at 18	1.99	1.94	11.12	13.16		0.024	0.007			
1144	do.	Oct. 20	18.04	1.57	4.1	—	9.1 at 22	3.81	3.70	9.21	13.11	0.90	0.024	0.007	0.422	0.324	
1165	do.	Nov. 18	17.96	1.46	4.09	—	9.1 at 20.8	2.34	1.96	10.64	12.71	0.98	0.023	0.008	0.419	0.292	
1179	do.	Dec. 1	18.74	1.46	4.03	—	9.7 at 21	2.27	2.15	11.38	13.55	1.02	0.023	0.008	0.422	0.292	
1146	Ben Davis	Oct. 23	16.63	1.42	6.3	—	8.8 at 25.4	3.67	3.74	7.32	11.26	1.09	0.025	0.008	0.329	0.231	
1164	do.	Nov. 18	16.92	1.40	5.3	—	9.1 at 20.8	2.88	2.68	7.84	11.27	1.25	0.029	0.008			
1176	do.	Dec. 1	15.15	1.40	5.3	—	9.1 at 21	2.50	3.78	7.34	11.02						
1185	do.	Dec. 9	16.13	1.39	6.8	—	8.8 at 18	1.50	1.43	8.31	9.81						

^a Starch determinations reduced by 0.5 per cent.^b As potassium carbonate.^c Starch absent after this date.^d Not used in calculations.

DISCUSSION OF ANALYTICAL DATA.

By referring to the graphic chart of Rhode Island Greening apples (fig. 1) it will be noted that the examination made on August 25 gave a sucrose content of 13 per cent on the basis of total solids. The content of sucrose increased steadily until about November 7, when the maximum was reached. During this time the content of starch had decreased, and the curve representing the decrease of starch is almost the reverse of the curve representing the increase of sucrose. The sucrose reached the maximum on the date mentioned, November 7, after which it rapidly decreased, and the date of the disappearance of the starch was almost, if not quite, coincident with that of the maximum content of sucrose.

The entire crop of apples was picked between October 6 and November 7, and unfortunately during that period, owing to the congested condition of the railroads, it was impossible to obtain samples of fruit, and the changes of composition taking place just at the time of ripening were not studied in as great detail as had been planned. It is probable that after picking the changes of composition may have progressed more rapidly than would have been the case if they could have been left on the tree.

The sum of the starch and sucrose (starch calculated as sucrose) is shown to decrease up to the point where the starch entirely disappeared. After this time the sucrose content decreases much more rapidly. The content of invert sugar increases from the earliest examination throughout the experiment. The curve representing the content of invert sugar is approximately the reverse of the curve representing first the decrease of starch and sucrose, and later the decrease of sucrose.

It will also be noted on the chart (fig. 1) that the total content of sugar (calculated as invert sugar) increased from the first examination to the date of the disappearance of the starch. After this date the curve representing the total sugar as invert merges with the curve representing the total carbohydrates as invert sugar. This latter curve does not include the cellulose. In the Rhode Island Greening apples the total carbohydrate content decreased to some extent after the disappearance of the starch. In the cases of the other varieties of apples previously mentioned, and some varieties to be described later, the percentage of total carbohydrates did not materially change during the later stages of the growth and development of the fruit.

At the same time a slight increase of carbohydrates expressed in terms of total solids seemed to occur during the growth of the apple before its separation from the tree, and a decrease occurred after picking, especially during long storage. Up to the point of the maximum starch content the percentage of total carbohydrates increases rapidly. This point is discussed in greater detail subse-

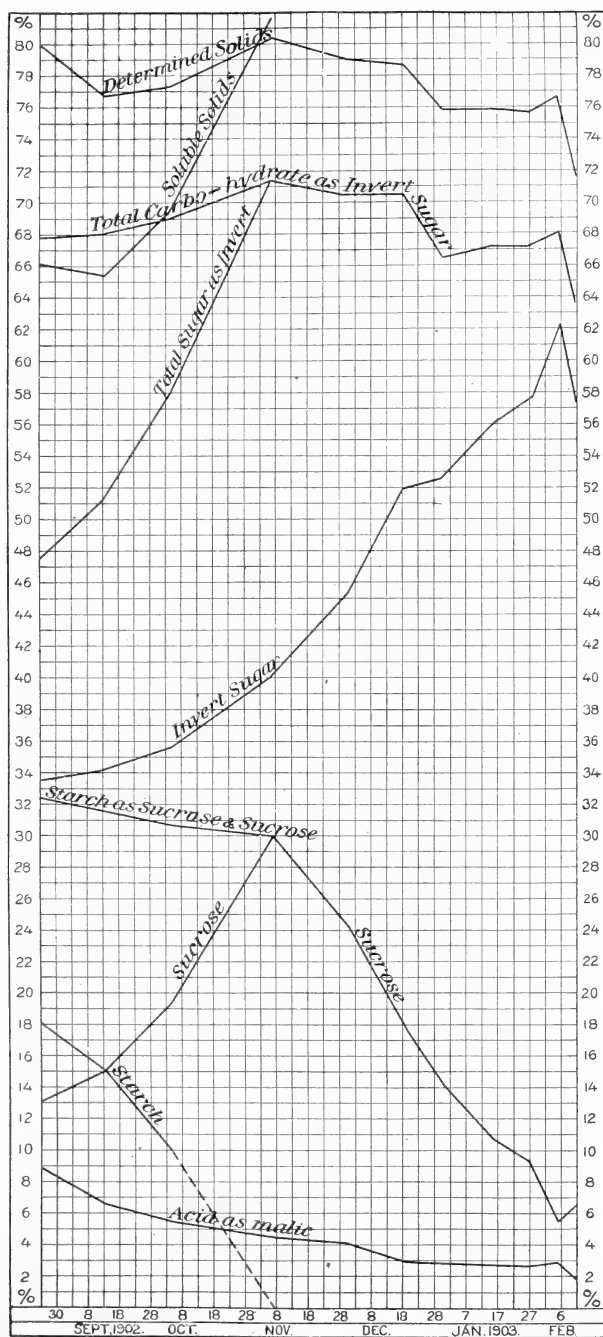


FIG. 1.—Chart showing results of analyses of Rhode Island Greening apples in common storage at room temperature (total solids basis).

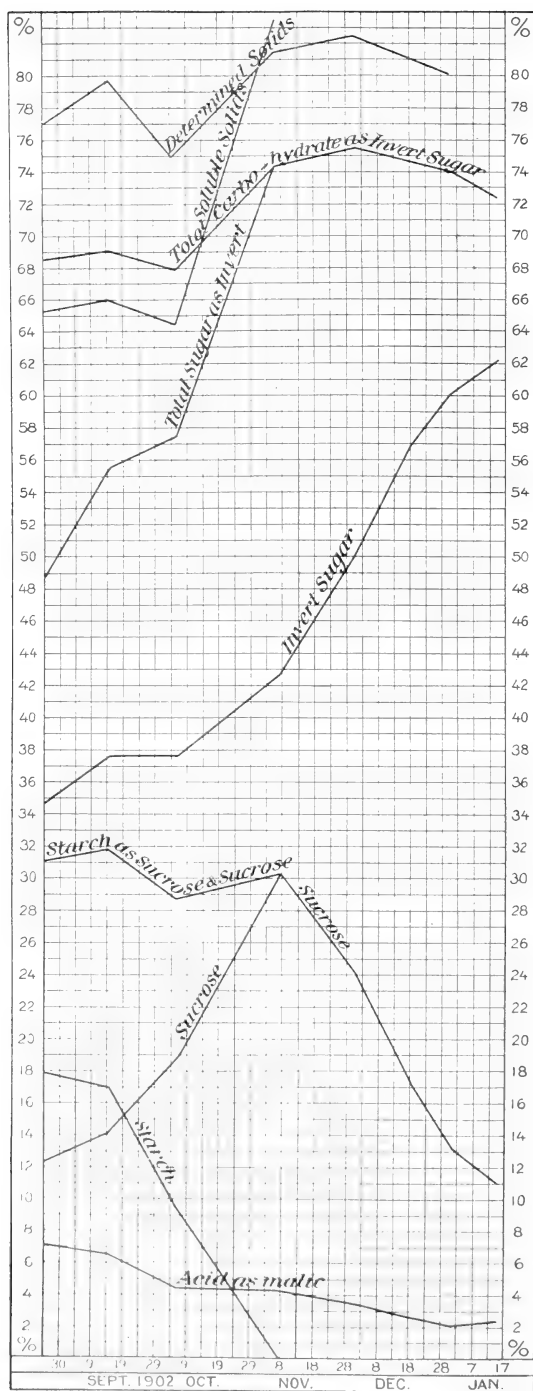


FIG. 2.—Chart showing results of analyses of Northern Spy apples in common storage at room temperature (total solids basis).

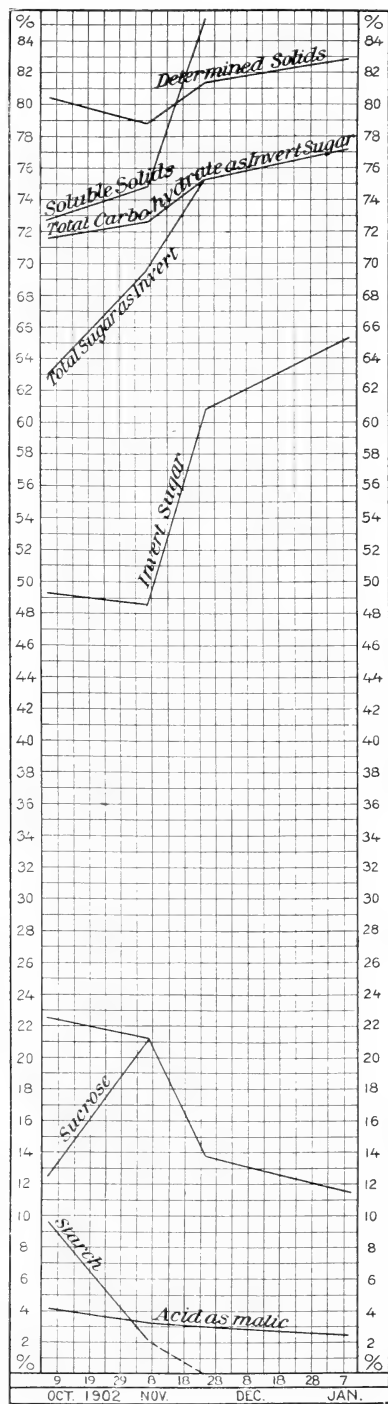
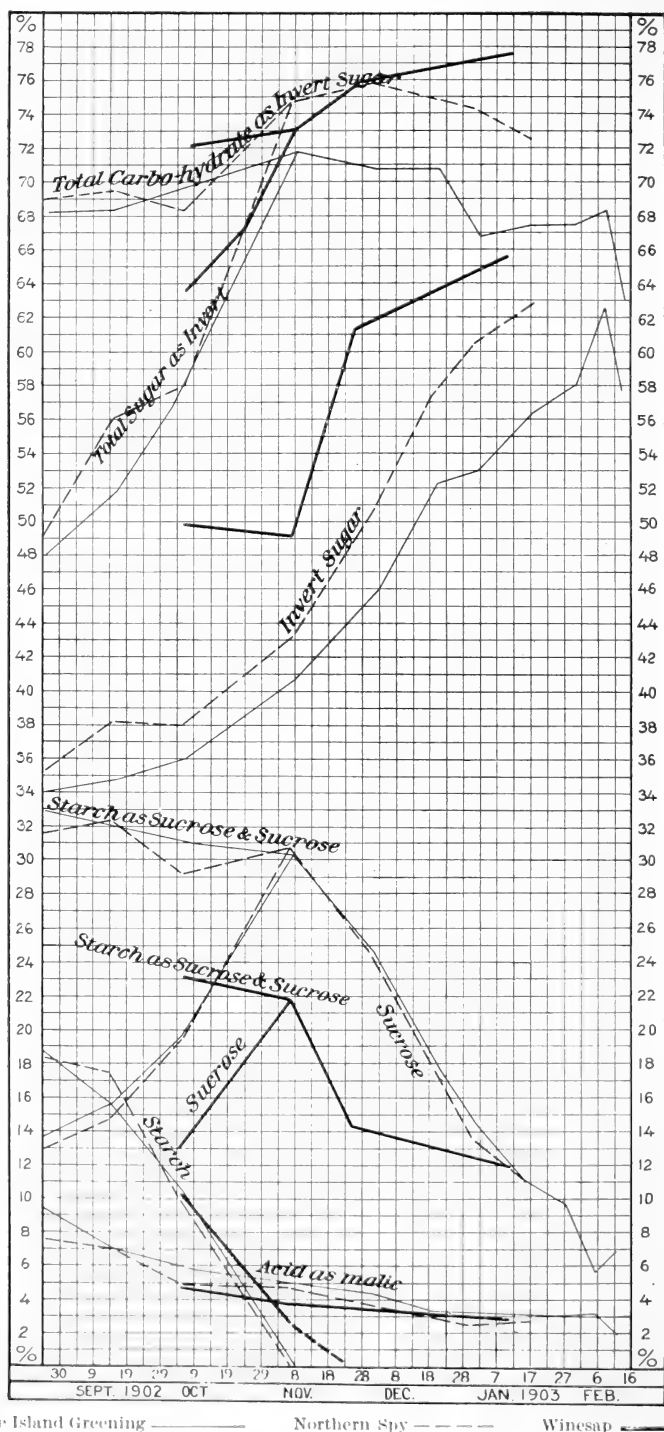


FIG. 3.—Chart showing results of analyses of Winesap apples in common storage at room temperature (total solids basis).



Rhode Island Greening

Northern Spy

Winesap

FIG. 1.—Chart comparing results of analyses of three varieties of apples in common storage at room temperature (total solids basis).

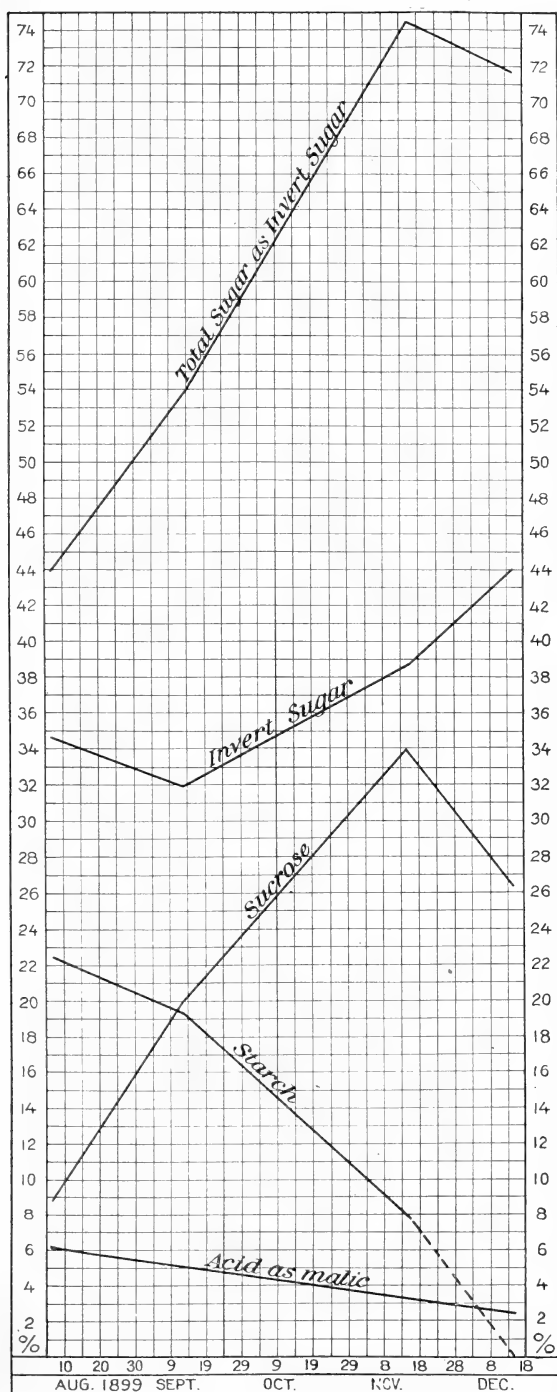


FIG. 5.—Chart showing results of analyses by C. A. Browne, jr., of Baldwin apples in common storage at room temperature. (Calculated to total solids basis.)

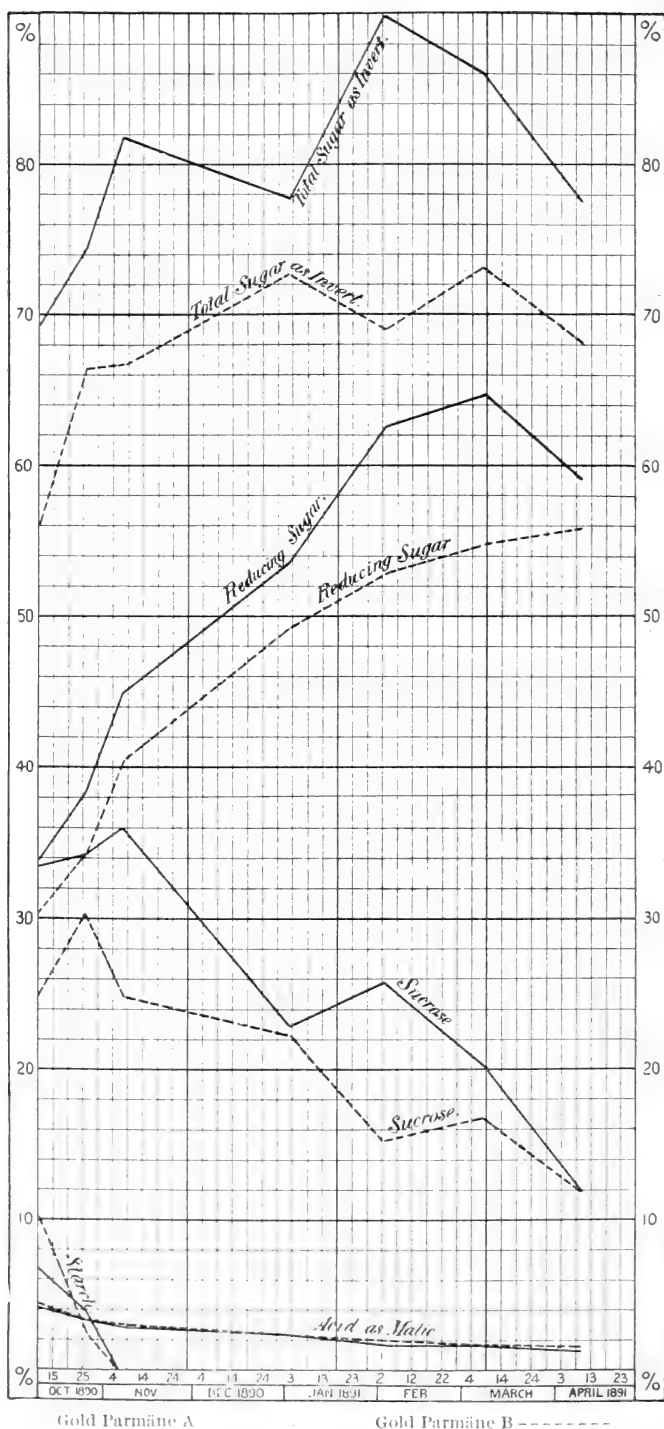


FIG. 6.—Chart showing results of analyses by Kulisch of apples stored at cellar temperature. (Calculated to an assumed basis of 17 per cent of total solids.)

quently with reference to immature fruit (see page 52). It is greatly to be regretted that this study was not begun at an earlier date, when the apples were less mature. Apparently they had reached, or perhaps passed, the maximum starch content before the first sample was secured, and a complete study of the life history of the fruit of course could not be made with the material taken. Certain irregularities in these results are due to the fact that the apples were grown at some distance from Washington, and two or three days elapsed from the time they were picked until they were used in analysis, these conditions not having been sufficiently understood when the work was begun. Further information on this subject is found on page 50. The acid content of the apple (on the percentage basis) decreased perceptibly throughout the experiment.

The foregoing comments on Rhode Island Greening apples are equally applicable to the results on the Northern Spy and the Winesap apples, platted in figures 2 and 3. The similarity of the curves representing the content of each ingredient is strikingly brought out in figure 4, in which the three varieties are compared.

For further comparison with these results those obtained by Dr. C. A. Browne, jr.,^a on Baldwin apples have been calculated to total solids and are presented in graphic form in figure 5. The results obtained by Kulisch,^b who worked with apples stored at cellar temperature, have also been recalculated, using an assumed total solid basis of 17 per cent, and the results are presented graphically in figure 6.

It will be noted that in all respects the results of Browne and of Kulisch are analogous to those obtained in this Bureau. Doctor Browne's experiment, however, was begun with apples in a more immature state than was the case with the work done in the Bureau of Chemistry, and for that reason more complete results were obtained, although his first examination also was made after the point of maximum starch content was passed. The work of Kulisch was carried on with two samples of apples of the same variety from the same orchard, but picked from different trees. His results show what wide variations in chemical composition may occur in fruit of the same variety grown under the same cultural conditions but on different trees.

THE RIPENING OF APPLES IN COLD STORAGE.

Late in August, 1902, an experimental export shipment was made of early-picked Ben Davis and Winesap apples from southern Illinois for the purpose of determining whether it would be profitable to place these apples on the London market so early in the season. The fruit had been picked about August 15. Some weeks later samples of both

^a Pa. State Dept. of Agr., Bul. No. 58.

^b Landw. Jahrb., 1892, 21: 871.

varieties were secured from lots from the same locality, picked at the same time, which had been kept in cold storage in Chicago. Samples were also secured of the same varieties picked at the usual time, about September 15, from trees in the same locality and situated similarly to those from which the export shipments had been taken. Early in October two barrels of each variety from each picking were shipped to Washington from Chicago in refrigerator cars. By special arrangement the apples were sent direct from cold storage to the cars and from the cars to the cold-storage rooms in Washington without exposing them to a higher temperature for more than a few moments at a time.

It is regretted that samples of the early-picked apples were not obtained at the time of picking, but owing to the commercial importance of the shipment in question it seemed advisable to work with this special lot. The apples were received on October 10 and were examined then and at varying intervals afterwards.

RESULTS OF ANALYSES.

The analytical results are found in Table III, and the platted data in figures 7, 8, 9, and 10. The date of the last examination reported in this bulletin was April 27, 1904. It is improbable, however, even if they had been made, that continued analyses would have been of any material value since the apples of all varieties were decaying rapidly. Owing to this fact it is suggested that the results of such analyses would lead to incorrect conclusions regarding the change in composition of apples in storage, because of a probable selective tendency of the rot, it being possible that apples of a certain composition may decay more rapidly than those of a different kind. At the same time no law has been discovered which seems to control or regulate the development of the decay in fruit. The selective tendency of decay is suggested, therefore, as a possibility which might lead to erroneous results in the work, and not as a probability indicated by the work itself. At the same time it is worthy of comment that after October 21, 1903, when decay began, the change of composition of the apples in storage which remained sound was noticeably different from the change occurring prior to that date.

With reference to the solids, the carbohydrates tended to increase after October 21, in the case of the early-picked Ben Davis and the late-picked Ben Davis and Winesap apples. The change in content of carbohydrates was more gradual in the case of the early-picked Winesap.

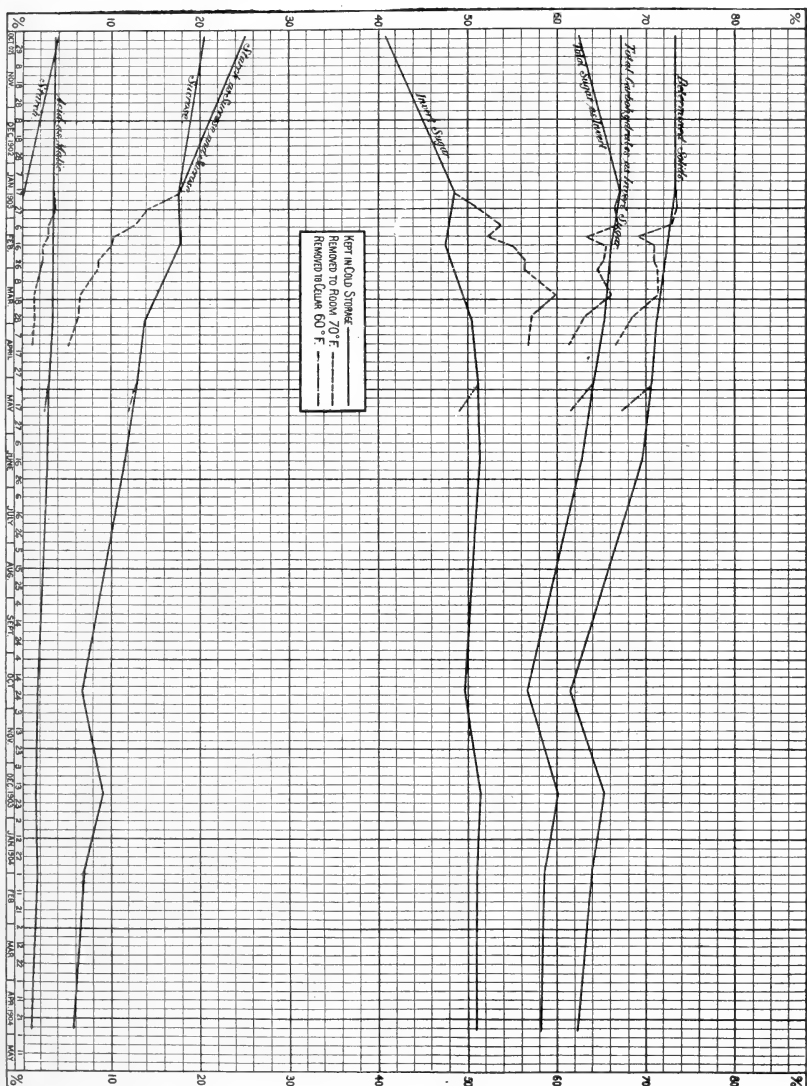


FIG. 7.—Chart showing results of analyses of Ben Davis apples picked August 15, 1902, and kept under varying conditions of storage (total solids basis).

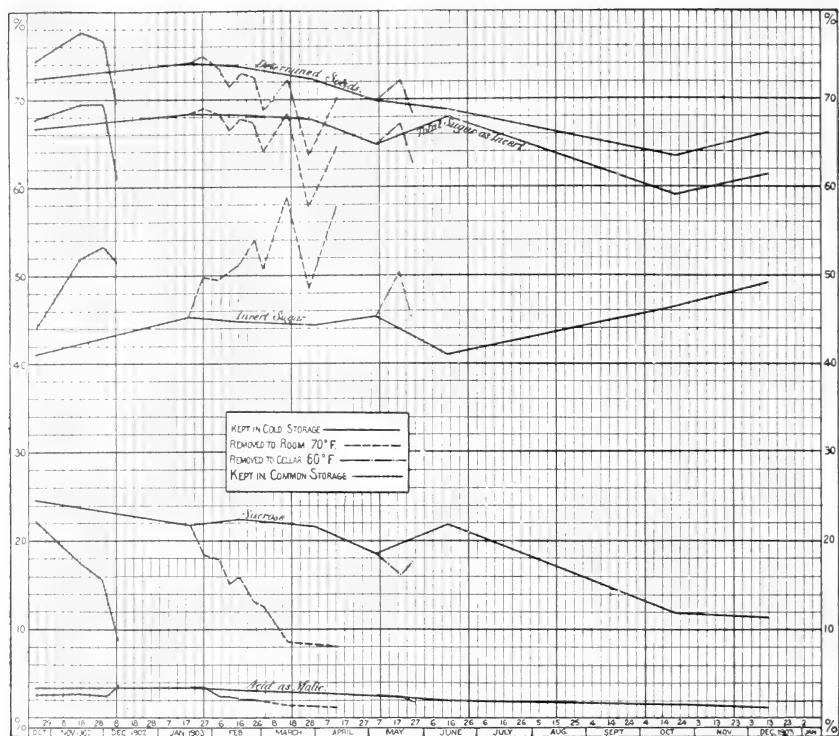


FIG. 8.—Chart showing results of analyses of Ben Davis apples picked September 15, 1902, and kept under varying conditions of storage (total solids basis).

TABLE III.—Analyses of cold-storage apples.

BEN DAVIS, PICKED AUGUST 15, 1902.

Serial No.	Date of analysis.	Total solids.	Acid as malic.	Starch. ^a	Polarization.			Sucrose.		Reducing sugar as invert.	Total sugar as invert.
					Before inversion.	After inversion.		By polarization.	By reduction.		
		Per cent.	Per cent.	Per cent.	° V.	° V.	° C.	Per cent.	Per cent.	Per cent.	Per cent.
1117	1902, Oct. 10	17.90	0.684	0.75	-3.3	-8.1	at 23.4	3.67	3.69	7.32	11.20
	1903,										
2148	Jan. 19	17.02	.589	None.	4.1	-8.3	at 24	2.99	2.99	8.27	11.42
2883	Feb. 16	17.41	.592	None.	-4.3	-8.4	at 21	3.12	3.07	8.27	11.50
4516	Mar. 31	16.60	.543	None.	-4.5	-7.37	at 25.7	2.22	2.33	8.38	10.83
5992	May 5	16.83	.482	None.	-4.8	-7.75	at 25	2.27	2.04	8.61	10.76
6189	June 15	16.92	.470	None.	-5.05	-7.7	at 25.7	2.05	1.81	8.70	10.60
7290	Oct. 21	16.80	.258	None.					1.11	8.34	9.51
7329	Dec. 17	16.86	.240	None.	-5.8	-7.9	at 22	1.61	1.42	8.64	10.13
	1904,										
7354	Jan. 29	15.97	.240	None.	-5.8	-7.37	at 22	1.20	1.04	8.26	9.37
1009	Apr. 27	16.21	.190	None.					.95	8.33	9.40

BEN DAVIS, PICKED SEPTEMBER 15, 1902.

1118	1902, Oct. 10	16.99	0.570	0.50	-2.8	-8.3	at 23.4	4.20	4.16	6.96	11.34
	1903,										
2150	Jan. 19	16.52	.543	None.	-3.9	-8.6	at 24	3.61	3.62	7.49	11.30
2884	Feb. 16	17.25	.520	None.	-3.7	-8.8	at 21	3.87	3.84	7.72	11.76
4517	Mar. 31	16.99	.459	None.	-3.5	-8.14	at 25.7	3.59	3.77	7.53	11.50
5993	May 5	16.79	.399	None.					3.11	7.62	10.89
6190	June 15	17.22	.322	None.	-4.8	-8.8	at 24.8	b 3.10	4.43	7.05	11.71
7291	Oct. 21	17.19	.231	None.					2.05	8.04	10.20
7330	Dec. 17	16.20	.180	None.	-5.3	-7.81	at 22	1.91	1.75	8.15	9.99

^aStarch determinations reduced by 0.5 per cent.

^bNot used in calculation.

TABLE III.—*Analyses of cold-storage apples—Continued.*

WINESAP, PICKED AUGUST 15, 1902.

Serial No.	Date of analysis.	Total solids.	Acid as malic.	Starch.	Polarization.			Sucrose.		Reducing sugar as invert.	Total sugar as invert.
					Before inversion.	After inversion.		By polarization.	By reduction.		
		<i>Per ct.</i>	<i>Per ct.</i>	<i>Per cent.</i>	$^{\circ}$ V.	$^{\circ}$ V.	$^{\circ}$ C.	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>
1143	1902. Oct. 20 1903.	17.52	0.663	1.00	-3.8	-7.5	at 22	2.82	2.63	8.72	11.49
2149	Jan. 19	17.06	.569	None.	-4.8	-7.25	at 24	1.88	1.74	9.88	11.71
2885	Feb. 16	17.08	.572	None.	-5.1	-7.4	at 21	1.76	1.68	10.07	11.84
4518	Mar. 31	17.07	.486	None.	-5.4	-7.4	at 25.7	1.56	1.84	10.28	12.22
5994	May 5	17.35	.469	None.	-----	-----	-----	-----	1.23	10.36	11.65
6491	June 15	17.62	.459	None.	-6.0	-7.6	at 26	1.24	1.01	10.85	11.91
7292	Oct. 21	16.34	.376	None.	-----	-----	-----	-----	.57	9.65	10.35
7331	Dec. 17 1904.	16.31	.380	None.	-6.6	-7.7	at 22	-----	.64	9.98	10.65
7355	Jan. 29	16.81	.310	None.	-6.4	-7.48	at 22	-----	.52	10.08	10.63
11010	Apr. 27	16.34	.300	None.	-----	-----	-----	-----	-----	9.90	-----

WINESAP, PICKED SEPTEMBER 15, 1902.

1142	1902. Oct. 20 1903.	16.84	0.684	0.86	-3.1	-7.2	at 22	3.12	3.47	8.01	11.66
2151	Jan. 19	16.65	.610	None.	-4.3	-7.5	at 24	2.46	2.51	9.28	11.92
2886	Feb. 16	17.10	.611	None.	-4.75	-8.25	at 21	2.68	2.48	9.51	12.12
4519	Mar. 31	17.03	.537	None.	-5.05	-8.14	at 25.7	2.39	2.53	9.96	12.62
5995	May 5	17.63	.549	None.	-5.2	-8.1	at 25	2.24	2.14	10.03	12.28
6492	June 15	16.87	.476	None.	-5.5	-7.6	at 26	1.63	1.51	10.46	12.05
7293	Oct. 21	16.77	.442	None.	-----	-----	-----	-----	.74	9.65	10.43
7332	Dec. 17 1904.	16.89	.440	None.	-6.8	-8.2	at 22	1.07	.97	10.14	11.16
7356	Jan. 29	16.33	.31	None.	-6.1	-7.37	at 22	.97	.75	10.12	10.95
11011	Apr. 27	16.60	.32	None.	-----	-----	-----	.84	.84	10.60	11.49

 α Not used in calculations.

When these four lots of apples were received, only a small amount of starch was present in the early-picked samples and in the late-picked Winesap, while the starch had entirely disappeared from the late-picked Ben Davis. On the date of the second examination, January 19, 1903, the starch in all the samples had entirely disappeared.

RESULTS WITH SAMPLES TAKEN OUT OF COLD STORAGE.

On this date samples were removed from cold storage and kept in a dry room in the laboratory at a temperature of about 70° F., where, owing to the dryness of the atmosphere, they lost their moisture content rapidly; before they were finally discarded some apples were evidently drier than others, and in many cases while one side of the apple was firm and plump the other was dry and shriveled. On this account great care in sampling was necessary and in spite of all the precautions taken the variations in composition were greater than was desirable. It was necessary also to take larger samples for analysis than would otherwise have been used.

TABLE IV.—*Analyses of apples removed from cold storage and held in common storage.*

BEN DAVIS, PICKED AUGUST 15, 1902.

Serial No.	Date of analysis.	Total solids.	Acid as malic.	Starch.	Polarization.			Sucrose.		Reducing sugars as invert.	Total sugars as invert.
					Before inversion.	After inversion.		By polarization.	By reduction.		
	1903.	Per cent.	Per cent.		° F.	° F.	° C.	Per ct.	Per ct.	Per ct.	Per ct.
2148	Jan. 19	17.02	0.589	None.	-4.4	-8.3	at 24	2.99	2.99	8.27	11.42
2614	Jan. 27	16.91	.600	None.	-3.9	-8.7	at 21	3.641	2.42	8.71	11.26
2715	Feb. 5	17.67	.482	None.	-5.85	-8.9	at 22	2.34	2.18	9.51	11.81
2826	Feb. 11	17.91	.506	None.	-5.9	-8.3	at 25	1.85	1.88	9.34	11.33
2891	Feb. 17	17.47	.372	None.	-6.5	-8.8	at 21	1.74	1.72	9.64	11.45
3531	Feb. 25	18.48	.405	None.	-6.7	-8.8	at 23.5	1.61	1.55	10.44	12.07
3578	Mar. 2	18.71	.311	None.	-6.5	-8.74	at 23.5	1.72	1.45	10.56	12.09
3724	Mar. 16	19.55	.250	None.	-8.4	-10.2	at 21.3	1.39	1.18	11.69	12.93
4464	Mar. 28	20.26	.255	None.	-8.25	-10.1	at 22.2	1.41	1.14	11.59	12.79
4659	Apr. 13	21.34	.218	None.	-8.5	-10.12	at 22.5	1.23	.93	12.12	13.09

BEN DAVIS, PICKED SEPTEMBER 15, 1902.

	1903.										
2150	Jan. 19	16.52	0.543	None.	-3.9	-8.6	at 24	3.61	3.62	7.49	11.30
2615	Jan. 27	16.69	.519	None.	-5.0	-8.8	at 21	2.88	a 3.48	7.83	a 11.49
2716	Feb. 5	17.37	.426	None.	-5.0	-9.1	at 22	3.14	3.08	8.59	11.83
2827	Feb. 11	17.29	.401	None.	-4.9	-8.3	at 25	2.62	a 2.48	8.72	a 11.33
2892	Feb. 17	18.10	.356	None.	-5.8	-9.7	at 21	2.94	2.84	9.31	12.29
3532	Feb. 25	18.27	.353	None.	-6.2	-9.4	at 23.5	2.45	2.32	9.87	12.31
3579	Mar. 2	18.53	.325	None.	-5.0	-7.77	at 21	2.28	2.40	9.37	11.92
3725	Mar. 16	19.54	.260	None.	-7.75	-9.79	at 21.5	1.55	1.78	11.52	13.39
4465	Mar. 28	20.92	.251	None.	-7.4	-10.1	at 22.2	2.06	1.85	10.16	12.10
4660	Apr. 13	20.77	.228	None.	-8.5	-11.0	at 22.5	1.91	1.38	11.96	13.44

WINESAP, PICKED AUGUST 15, 1902.

	1903.										
2149	Jan. 19	17.06	0.569	None.	-4.8	-7.25	at 24	1.88	1.74	9.88	11.71
2616	Jan. 27	17.77	.574	None.	-5.5	-8.1	at 21	1.97	1.77	10.70	12.56
2717	Feb. 5	18.35	.413	None.	-6.45	-8.5	at 22	1.57	1.47	11.69	13.24
2828	Feb. 11	18.13	.450	None.	-5.8	-8.0	at 25	1.69	1.19	11.15	12.40
2893	Feb. 17	17.40	.412	None.	-6.3	-7.9	at 21	1.23	1.13	10.99	12.18
3533	Feb. 25	18.87	.365	None.	-6.8	-8.3	at 23.5	1.19	1.01	12.19	13.25
3580	Mar. 2	18.44	.375	None.	-6.25	-7.6	at 23.5	1.11	.89	11.58	12.52
3726	Mar. 16	19.09	.340	None.	-7.05	-6.82	at 21.574	11.82	12.59
4466	Mar. 28	20.88	.281	None.	-7.9	-9.35	at 22.2	1.10	.83	12.51	13.38
4661	Apr. 13	20.79	.250	None.	-7.95	-9.57	at 22.5	1.21	.83	13.13	14.01

WINESAP, PICKED SEPTEMBER 15, 1902.

	1903.										
2151	Jan. 19	16.65	0.610	None.	-4.3	-7.5	at 24	2.46	2.51	9.28	11.92
2617	Jan. 27	17.29	.643	None.	-4.7	-8.05	at 21	2.54	2.51	9.81	12.45
2718	Feb. 5	17.83	.543	None.	-5.4	-8.50	at 22	2.38	2.11	10.63	12.85
2829	Feb. 11	18.41	.514	None.	-5.8	-8.7	at 25	2.23	a 1.71	10.92	a 12.74
2894	Feb. 17	18.50	.424	None.	-6.5	-9.07	at 21	1.95	13.64
3534	Feb. 25	18.75	.415	None.	-6.3	-8.83	at 23.5	1.94	1.59	11.86	13.53
3581	Mar. 2	18.29	.385	None.	-6.1	-8.3	at 23.5	1.68	1.35	11.05	12.47
3727	Mar. 16	19.69	.310	None.	-7.4	-8.53	at 21.5	.85	1.29	12.19	13.55

a Not used in calculations.

The results of the analyses of the apples removed from cold storage and kept in the laboratory appear in Table IV, and they are shown graphically in figures 7, 8, 9, and 10. In spite of the irregularity of the curves it will be noticed that the apples ripened much more rapidly than those kept in cold storage, and that, before the samples were

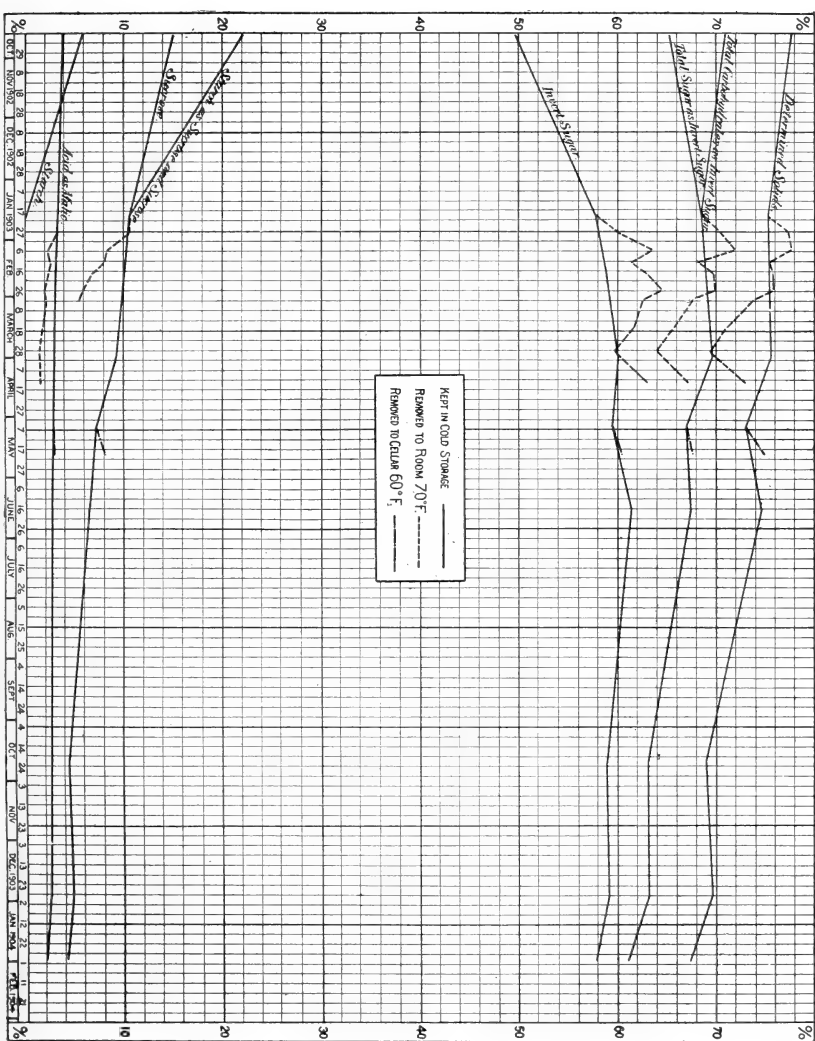


FIG. 9.—Chart showing results of analyses of Winesap apples picked August 15, 1902, and kept under varying conditions of storage (total solids basis).

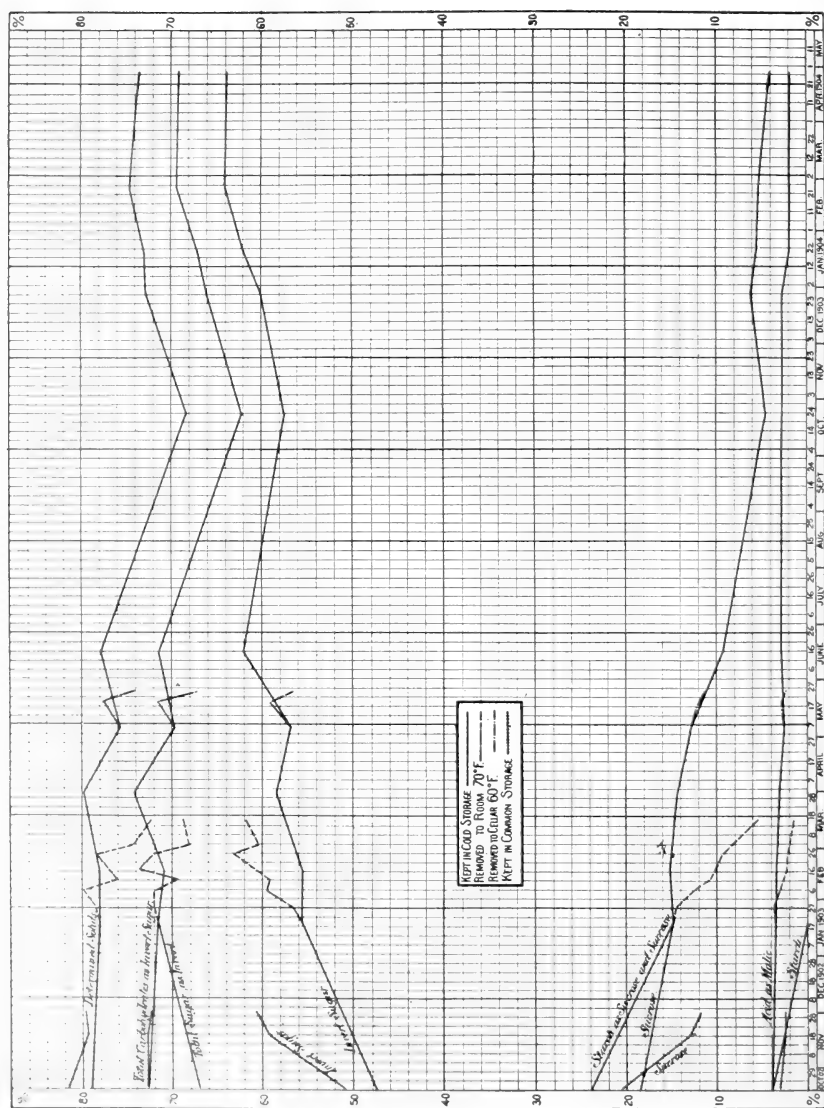


FIG. 10.—Chart showing results of analyses of Winesap apples picked September 15, 1902, and kept under varying conditions of storage (total solids basis).

destroyed by rot, they had closely approximated the composition attained by the apples in cold storage some months later. That is, the percentage of total sugars expressed as invert sugar and of acids expressed as malic acid reached about the same figure in those kept in the laboratory as was reached at a later date in those kept in cold storage. In one respect, however, the composition of the Ben Davis stored in the laboratory changed in a manner very different from that of the sample kept in cold storage: In the former the sucrose decreased before April 13, 1903, until it reached a lower figure for sucrose than was found for the cold storage apples on April 27, 1904, more than twelve months later. The apples ripened in the laboratory contained a higher percentage of invert sugar and a lower percentage of cane sugar than those ripened in cold storage at the time when each of them was rendered useless by decay. In the case of the Winesap apples, however, this difference in the changes in sucrose and invert sugar does not obtain. Approximately the same minimum for the sucrose was reached in the apples stored in the laboratory as in those in cold storage, the latter having reached a minimum sucrose content about October 21, 1903, approximately seven months after the laboratory sample had been rendered useless by decay.

It appears, so far as can be determined from this work, that the changes in composition (the content of starch, sugar, and acids) in cold storage do not greatly differ from those which occur in common storage, the chief difference being in the rapidity with which the changes take place. At the same time, the fact that the changes which take place in storage at ordinary temperatures give higher maximum values for invert sugar and lower minimum values for sucrose in some instances than those occurring in cold storage is worthy of consideration and further study. As an illustration of this may be noted the scalding of apples in cold storage. Scald is probably caused or accompanied by a chemical change, but as yet this can not be demonstrated by chemical analysis.

On May 5, 1903, samples were removed from cold storage and placed in a cellar temperature at about 60° F. Only one subsequent analysis of the apples so stored was made. They remained in good condition during the greater part of the summer, keeping much better than other cold storage apples bought on the market and removed to the same cellar and better than some apples removed directly from the trees to the cellar. The apples of both varieties picked on August 15, 1902, remained firm and in good condition until the latter part of July, 1903, and the last of them gave way to decay about the 1st of October, 1903.

It was noticeable that a change in the ripening of all varieties occurred about October 1, 1903. After that period the ripening progressed somewhat more slowly than before. At the same time the apples

which previously had been sound now began to decay. It is suggested that the two conditions may be due to the same cause; that is, the loss of vitality of the apples may have exposed them to decay and rot, or at least made them less resistant to rot, and at the same time may have led to slower changes in the composition of the fruit. As has been suggested in other connections, the apparent retarding in the ripening process may have been due to the greater susceptibility to decay of the ripest apples, and consequently to an increase in the percentage of the relatively greener apples on each successive examination.

THE RESPIRATION OF APPLES IN COMMON AND COLD STORAGE.

On October 20, 1902, a barrel of Ben Davis apples, grown at South Onondaga, N. Y., was secured for respiration experiments.

DESCRIPTION OF APPARATUS AND METHODS EMPLOYED.

Three stone jars of the form shown in figure 11 were secured as containers. These jars were the ordinary glazed stoneware chlorin

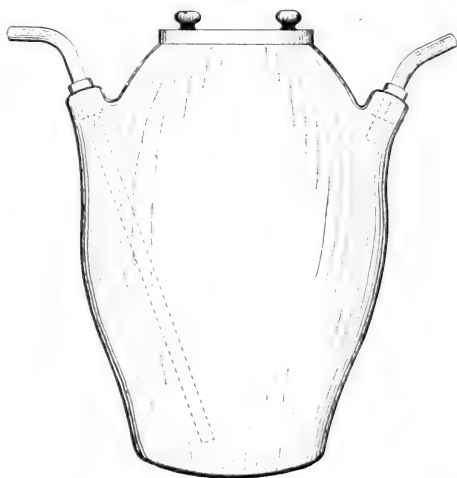


FIG. 11.—Jar used in respiration experiments.

generators with stoneware covers having a ground joint. As shown in the illustration, the jars were arranged to afford the passage of a slight current of air. A guard tube of soda lime, then a drying tube of calcium chlorid, and then a tube of moist pumice stone were connected in series before the apparatus. The last-named tube was for the purpose of moistening the air which passed through the apparatus, as the passage of a dry current of air would desiccate the apples and cause them to shrink abnormally. The tube of calcium chlorid just before the pumice stone was used in order that by repeated weighings of the latter the amount of water carried over into the jar with the current of air might be determined. Connected in series after the generator were

two calcium-chlorid tubes followed by two soda-lime tubes. The end of the second soda-lime tube contained calcium chlorid to prevent the loss of water from the soda lime. Finally there was a guard tube of soda lime. The current of air for this purpose was afforded by two bottles, one of which was filled with water and stood on a table, while the other was empty and was placed on the floor. Each bottle was

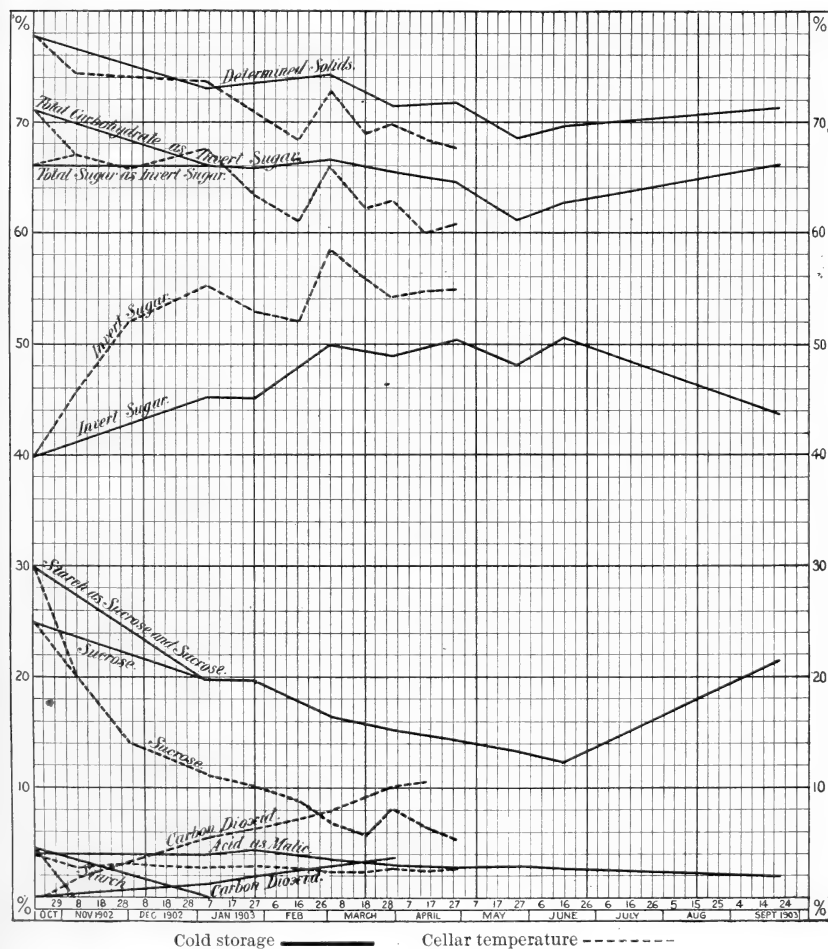


FIG. 12.—Chart comparing results of analyses of Ben Davis apples used in respiration experiments and kept in cold storage and at cellar temperature (total solids basis).

closed with a two-holed stopper. A tube connected the bottom of the first bottle with the second, and each bottle was connected with one end of the train of absorption tubes, the full bottle acting as an aspirator, while the empty one supplied the pressure. The current of air entered the jar through a delivery tube, passed to the bottom of the jar, and issued from a delivery tube leading from the top. The three

jars were weighed and filled with apples taken from the same lot. One of the jars was placed in cold storage at a temperature of about 0° C. (32° F.); the second jar was placed in a cellar temperature of about 60° F.; the third jar was also placed in cold storage, but, instead of being supplied with a current of air, it was sealed and not opened until September 19, 1903, when an analysis of the contents of the jar, both the apples and the gas, was made.

In the case of the fruit in cold storage difficulty was experienced in several instances when the temperature read slightly below the freezing point, the water freezing in the tube connecting the two bottles. This difficulty was overcome by dissolving salt in the water employed to furnish a current of air.

RESULTS OF RESPIRATION EXPERIMENTS.

The results of the examination of the apples employed for the respiration experiment are given in the graphic chart shown in figure 12 and in Table V. These results coincide in a general way with the experiments in common storage and in cold storage already described. The fruit kept at a higher temperature ripened much more rapidly than that kept in cold storage. Moreover, the sucrose reached a lower minimum and the invert sugar a higher maximum in the experiments conducted at the higher temperature. These facts also confirm those obtained in earlier experiments. As in the case of other experiments previously described (p. 39), the sucrose of the apples kept in cold storage reached a minimum about the same time (in this case June 16, 1903) that the invert sugar of the same apples reached a maximum. After that date the percentage of sucrose increased and the percentage of invert sugar decreased. It is again suggested that this may be due to the selective tendency of the rot of the apples, whether the rot was due to bacteria, fungus growths, or to physiological death; and further, that the more mature apples may be more subject to decay than the less mature, whatever the cause of the decay.

During the progress of the experiments the carbon dioxide and water were determined at short intervals, usually daily, up to the time when the experiments at cellar temperature were discontinued owing to the exhaustion of the sample. At this time the apples in cold storage began to rot and the increased amount of carbon dioxide given out vitiated any conclusions which might have been drawn from the work had it been longer continued. The amounts of carbon dioxide given off from both jars are shown graphically in figure 12. On comparing the amount of carbon dioxide eliminated with the malic-acid curve it hardly seems possible that the carbon dioxide can be accounted for by the disappearance of malic acid, as several workers, notably Gerber (see p. 19), have considered to be the case. On the other hand, the curve representing the content of total carbohydrates,

TABLE V.—*Analyses of apples used in respiration experiment.*

BEN DAVIS AT CELLAR TEMPERATURE.

Serial No.	Date of analysis.	Total solids.	Acid as malic.	Starch. ^a	Polarization.		Sucrose.		Reducing sugar as invert.	Total sugar as invert.	Weight of apples.	Increase of carbon dioxide. ^b		Total carbon dioxide at date. ^c
					Before inversion.	After inversion.	By polarization.	By inversion.				Grams.	Per cent.	
	1902.				°V.	°C.								
1145	Oct. 20	15.40	0.630	0.71	-2.5	-7.6 at 21	3.89	3.83	6.14	10.17	16,020	43.651	0.2725	0.2725
1153	Nov. 7	14.88	.421	.00	-3.7	-7.7 at 21	3.02	3.01	6.81	9.98	12,940	39.197	.3029	.5754
1175	Dec. 1	14.14	.448	.02	-4.9	-7.7 at 21	2.12	1.88	7.34	9.31				
	1903.													
1851	Jan. 5	13.80	.394	-5.5	-7.9 at 21.5	c 1.82	1.55	7.62	9.28	11,125	24.367	.2191	.7944
2612	Jan. 27	13.85	.409	-5.1	-7.0 at 21	1.44	1.37	7.33	8.77	9,670	10,190	.1054	.8998
2887	Feb. 16	13.76	.328	-5.35	-7.04 at 21	1.28	1.17	7.16	8.39	8,250	11,081	.1343	1.0841
3583	Mar. 2	13.02	.387	-6.0	-7.45 at 23.5	1.10	.90	7.62	8.57	7,020	6,167	.0879	1.1220
3803	Mar. 18	13.78	.341	-6.2	-7.31 at 22.2	.79	.836	7.69	8.57	5,430	8,699	.1584	1.2804
4194	Mar. 30	12.58	.345	-5.3	-6.6 at 25.7	1.005	1.04	6.82	7.92	4,180	3,985	.0953	1.3757
4671	Apr. 14	13.59	.338	-6.2	-7.37 at 22.5	.89	.58	7.43	8.04	3,135	2,218	.0708	1.4465
5915	Apr. 28	13.33	.363	-6.25	-7.15 at 22	.69	.74	7.31	8.09				

BEN DAVIS AT 0° C. (32° F.).

1145	Oct. 20	15.40	0.630	d 0.71	-2.5	-7.6 at 22	3.89	3.83	6.14	10.17				
1852	Jan. 5	14.61	.596	-3.5	-7.4 at 21.5	2.96	2.83	6.60	9.63	17,800	41.664	0.2341	0.2341
2613	Jan. 27	15.13	.656	-3.1	-7.5 at 21	e 3.34	2.98	6.83	9.96	16,170	11,979	.0741	.3082
3583	Mar. 2	14.21	.506	-4.06	-7.21 at 23.5	2.45	2.25	7.09	9.46	14,900	19,070	.1280	.4362
4520	Mar. 31	14.09	.430	-4.1	-6.71 at 25.7	2.02	2.30	6.89	9.23	13,565	6,568	.0484	.4846
5914	Apr. 28	14.27	.393	-4.5	-7.37 at 22	2.19	1.92	7.19	9.21				
6250	May 26	14.67	.425	-3.9	-6.6 at 27	2.10	1.81	7.06	8.97				
6488	June 15	14.50	.385	-4.6	-7.04 at 24.8	1.89	1.68	7.32	9.09				
7211	Sept. 21	13.47	.269				2.88	5.88	8.90				

BEN DAVIS, SEALED JAR AT 0° C. (32° F.).

7202	Sept. 19	14.26	0.234	(d)	0.69	5.93	6.66
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^a Starch determinations reduced by 0.5 per cent.^b The figures in these columns show the weight and percentage of carbon dioxide formed between dates of analyses.^c The figures in this column show the percentage of the total carbon dioxide formed up to the date of analysis.^d Starch absent after this date.^e Not used in calculations.

expressed as invert sugar, is approximately the reverse of the curve representing the evolution of carbon dioxid. The probability that the carbon dioxid results from the decomposition of carbohydrate bodies is strengthened by the fact that after protracted storage the apple has apparently lost vitality, and changes in composition proceed much more slowly than in the early days of storage. It is probable that at this time the quantity of carbon dioxid given off by reason of the original transpiration of the sound apple would be considerably less than when chemical processes were more vigorous. At the same period of time the changes in the composition of carbohydrate bodies apparently proceed much more slowly than before, whereas with apples preserved in cold storage the change in the content of acid proceeds uniformly. The amounts of water aspirated into the jars were found to be uniformly larger than the amounts carried out by the current of air. This may be accounted for by the probable condensation of moisture on the interior of the jar, as this has been observed in later experiments.

THE GROWTH OF APPLES.

In the summer of 1903 the study of the growth of apples was taken up. It was desired to begin the work with the apples at the earliest stage practicable, but at the same time it seemed best to wait until the "June drop" was over, as the apples which did not cling to the trees might be different in composition from those which remained securely fastened. For this reason the apples when picked were not so small as had been planned for the earliest experiments. For the same reason, however, samples taken at the earlier date would probably have been of little or no value. As in the case of the experiments previously described, the apples were selected and furnished by the pomologist in charge of field investigations in the Bureau of Plant Industry, Mr. William A. Taylor. The fruits chosen were those which offered the widest possible range of characteristics within the number of samples which could be examined. (See Table I.)

RESULTS OF CONSECUTIVE ANALYSES OF GROWING APPLES.

Table VI shows the results of the analyses of apples at different times during the period of growth in terms of the original sample:

TABLE VI.—*Analyses of growing apples.*

SUMMER APPLES—EARLY STRAWBERRY.

Serial No.	Date of analysis.	Total solids.	Acid as malic.	Starch. ^a	Polarization.			Sucrose.		Reducing sugar as invert.	Total sugar as invert.	Weight per apple.
					Before inversion.	After inversion.		By polarization.	By reduction.			
	1903.	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per cent.</i>	° V	° V	° C	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Grams.</i>
6420	June 11	13.07	1.38	2.76	-3.2	-3.35	at 25	0.11	0.29	3.19	3.51	18.56
6519	June 17	14.11	1.42	3.37	-3.2	-3.96	at 26.2	.59	.69	3.38	4.11	23.5
<i>b 6751</i>	<i>June 23</i>	<i>13.65</i>	<i>1.08</i>	<i>.79</i>	<i>-2.85</i>	<i>-5.17</i>	<i>at 24</i>	<i>1.78</i>	<i>1.73</i>	<i>5.21</i>	<i>7.03</i>	<i>22.0</i>
6776	June 25	14.67	1.16	3.92	-3.60	-5.17	at 26	1.22	1.18	3.95	5.19	31.52
<i>b 6841</i>	<i>July 3</i>	<i>13.08</i>	<i>.....</i>	<i>.48</i>	<i>-3.50</i>	<i>-5.60</i>	<i>at 27</i>	<i>1.63</i>	<i>1.69</i>	<i>6.16</i>	<i>7.94</i>	<i>29.54</i>
6837	July 2	13.72	1.27	2.91	-3.70	-5.20	at 27	1.16	1.05	4.75	5.85	43.12
<i>b 6851</i>	<i>July 11</i>	<i>13.89</i>	<i>.....</i>	<i>.33</i>	<i>-3.30</i>	<i>-6.35</i>	<i>at 26</i>	<i>2.31</i>	<i>2.32</i>	<i>6.32</i>	<i>8.76</i>	<i>40.54</i>
6842	July 7	13.47	.98	2.34	-3.60	-5.65	at 30	1.61	1.64	4.93	6.66	47.06
6845	July 10	13.78	1.02	2.86	-4.35	-6.10	at 26	1.35	1.38	5.19	6.64	52.92
6856	July 15	13.62	.92	2.06	-4.5	-6.60	at 23.5	1.61	1.62	5.31	7.01	50.62
6863	July 17	14.16	.88	2.24	-4.0	-8.03	at 28	<i>c 3.14</i>	2.01	5.56	7.68	63.5
<i>b 6907</i>	<i>Aug. 5</i>	<i>13.39</i>	<i>.64</i>	<i>.00</i>	<i>-2.8</i>	<i>-6.16</i>	<i>at 27.6</i>	<i>2.63</i>	<i>2.40</i>	<i>5.76</i>	<i>8.38</i>	<i>58.06</i>
6883	July 22	14.20	.75	1.78	-3.0	-7.92	at 28	<i>c 3.83</i>	2.70	5.05	7.89	64.52
6888	July 24	14.84	.76	1.29	-2.4	-6.60	at 29	3.29	3.21	5.15	8.53	67.70
6895	July 29	14.82	.70	1.19	-2.4	-6.71	at 32	3.42	3.10	5.38	8.64	64.80
6901	Aug. 5	15.14	.76	.91	-1.4	-6.60	at 27.6	4.07	4.16	5.14	9.52	72.15

SUMMER APPLES—BOUGH.

6422	June 11	13.49	0.295	2.00	-3.4	-3.4 at 25	0.00	0.06	5.57	5.64	37.88
6520	June 17	13.66	.315	2.47	-3.75	-4.4 at 26.2	.5	.61	5.40	6.04	55.20
<i>b 6752</i>	<i>June 23</i>	<i>13.74</i>	<i>.295</i>	<i>.76</i>	<i>-3.0</i>	<i>-4.3 at 24</i>	<i>.99</i>	<i>.94</i>	<i>6.03</i>	<i>7.02</i>	<i>51.30</i>
6777	June 25	14.44	.248	2.52	-3.8	-5.65 at 26	1.43	1.27	5.83	7.17	75.34
<i>b 6840</i>	<i>July 3</i>	<i>13.95</i>	<i>.....</i>	<i>.43</i>	<i>-4.4</i>	<i>-5.65 at 27</i>	<i>.97</i>	<i>.99</i>	<i>7.57</i>	<i>8.58</i>	<i>64.00</i>
6838	July 2	14.27	.348	2.16	-3.3	-4.60 at 27	1.01	1.05	5.92	7.02	96.94
<i>b 6852</i>	<i>July 11</i>	<i>13.24</i>	<i>.....</i>	<i>.36</i>	<i>-4.15</i>	<i>-6.2 at 26</i>	<i>1.59</i>	<i>1.63</i>	<i>7.04</i>	<i>8.76</i>	<i>93.24</i>
6843	July 7	14.39	.178	1.79	-4.1	-5.55 at 30	1.14	1.40	6.22	7.69	104.00
6846	July 10	14.95	.211	2.00	-5.5	-6.85 at 26	1.04	1.10	7.06	8.22	97.30
6857	July 15	14.08	.194	1.49	-6.4	-8.25 at 23.5	1.42	1.29	7.41	8.77	101.20
6864	July 17	15.24	.275	1.52	-5.5	-8.35 at 28	<i>c 3.00</i>	1.98	7.15	9.23	113.10
<i>b 6908</i>	<i>Aug. 5</i>	<i>14.18</i>	<i>.301</i>	<i>.19</i>	<i>-3.4</i>	<i>-5.78 at 27.6</i>	<i>1.86</i>	<i>1.80</i>	<i>6.13</i>	<i>8.02</i>	<i>119.40</i>
6884	July 22	15.42	.181	2.13	-3.5	-5.40 at 28	1.48	1.69	5.15	6.93	108.60
6889	July 24	16.86	.188	1.10	-3.8	-7.10 at 29	2.58	2.53	6.88	9.54	126.30
6896	July 29	16.78	.164	2.43	-4.3	-7.26 at 32	2.25	1.82	6.86	8.78	98.64

SUMMER APPLES—YELLOW TRANSPARENT.

6421	June 11	11.46	1.77	1.49	-3.2	-3.85 at 25	0.50	0.38	3.70	4.10	30.54
6521	June 17	12.28	1.44	1.41	-3.2	-4.84 at 26.2	1.27	1.43	4.41	5.91	32.40
<i>b 6753</i>	<i>June 23</i>	<i>11.72</i>	<i>1.23</i>	<i>.30</i>	<i>-3.4</i>	<i>-5.06 at 24</i>	<i>1.28</i>	<i>1.37</i>	<i>4.99</i>	<i>6.43</i>	<i>31.95</i>
6778	June 25	12.28	1.22	1.49	-3.05	-5.65 at 26	2.01	1.98	4.50	6.58	57.95
<i>b 6839</i>	<i>July 3</i>	<i>11.45</i>	<i>.....</i>	<i>.12</i>	<i>-3.75</i>	<i>-5.9 at 27</i>	<i>1.67</i>	<i>1.63</i>	<i>6.27</i>	<i>7.97</i>	<i>48.30</i>
6836	July 2	12.96	1.27	1.46	-3.75	-6.35 at 27	2.02	2.11	4.45	6.67	76.52
<i>b 6850</i>	<i>July 11</i>	<i>12.21</i>	<i>.....</i>	<i>.04</i>	<i>-4.10</i>	<i>-6.55 at 26</i>	<i>1.89</i>	<i>1.86</i>	<i>6.24</i>	<i>8.20</i>	<i>74.50</i>
6844	July 7	12.65	1.00	.96	-4.25	-6.75 at 30	1.96	1.93	5.96	8.00	87.34
6847	July 10	13.18	.81	.60	-4.05	-7.10 at 26	2.36	2.37	6.18	8.67	91.78
6858	July 15	13.39	.96	.97	-4.30	-7.3 at 23.5	2.30	2.22	5.81	8.15	89.60
6865	July 17	13.90	.95	1.04	-3.70	-8.8 at 28	<i>b 3.97</i>	2.91	5.96	9.02	97.00
<i>b 6909</i>	<i>Aug. 5</i>	<i>13.40</i>	<i>.61</i>	<i>.09</i>	<i>-3.3</i>	<i>-6.38 at 27.6</i>	<i>2.41</i>	<i>2.76</i>	<i>5.88</i>	<i>8.78</i>	<i>89.75</i>
6885	July 22	13.85	.89	.87	-3.9	-8.25 at 28	3.39	3.33	5.27	8.78	112.7
6890	July 24	14.62	.94	.35	-3.2	-7.6 at 29	3.44	3.12	5.93	9.21	117.9
6894	July 29	13.99	.79	.53	-3.5	-7.04 at 32	2.81	2.74	6.01	8.89	99.36

^aStarch determinations reduced 0.5 per cent.

^bAll figures in italics give analyses of apples held in the ice box since the preceding date.

^cNot used in calculation.

TABLE VI.—Analyses of growing apples—Continued.

WINTER APPLES—BEN DAVIS.

Serial No.	Date of analysis.	Total solids.	Acid as malic.	Starch.	Polarization.		Sucrose.		Reducing sugar as invert.	Total sugar as invert.	Weight per apple.
					Before inversion.	After inversion.	By polarization.	By reduction.			
	1903.	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per cent.</i>	<i>° V.</i>	<i>° V</i>	<i>° C</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	<i>Grams.</i>
6493	June 16	13.63	1.64	2.23	-1.3	-2.0	at 25	0.54	0.49	2.35	3.87
6833	June 30	13.37	1.27	3.03	-1.55	-2.35	at 27	.62	.67	3.04	32.47
a 6855	July 13	13.5872	-1.8	-3.4	at 23.5	1.23	1.21	5.09	32.11
6891	July 28	15.71	.89	3.67	-2.65	-3.96	at 32	1.04	1.13	4.52	58.60
6923	Aug. 18	14.92	.78	3.16	1.46	4.36	95.4
7258	Sept. 24	15.05	.52	2.40	2.59	4.83	130.2
7285	Oct. 15	14.86	.52	1.46	3.13	5.30	167.9
7294	Oct. 23	14.8294	3.92	5.53	149.5
7303	Oct. 30	14.68	.43	.38	-1.7	-6.87	at 24.1	4.30	3.87	5.84	178.6
7308	Nov. 5	15.73	.41	-1.95	-7.15	at 23.7	3.99	3.71	5.83	147.4

WINTER APPLES—HUNTSMAN.

	1903.										
6494	June 16	12.75	1.43	1.55	-1.2	-1.76	at 25	0.43	0.47	2.54	15.04
6834	June 30	13.46	1.12	2.65	-1.1	-1.90	at 27	.62	.69	3.31	31.70
a 6854	July 13	12.9669	-1.7	-3.00	at 23.5	1.00	1.00	5.21	25.69
6892	July 28	15.33	.80	3.69	-2.0	-3.74	at 32	1.38	1.24	4.30	59.43
6924	Aug. 18	14.07	.60	2.69	1.78	4.08	93.50
7259	Sept. 24	14.03	.37	1.00	3.09	5.08	8.33
7286	Oct. 15	13.50	.40	.30	3.65	5.56	163.6
7295	Oct. 23	14.2372	4.82	4.98	10.05
7304	Oct. 30	13.08	.41	.19	+ .5	-4.9	at 24.1	4.49	4.26	4.82	9.11
7309	Nov. 5	13.39	.36	+ .3	-5.55	at 23.7	4.49	4.30	4.83	9.36

WINTER APPLES—WINTER PARADISE.

	1903.										
6495	June 16	13.57	0.38	1.98	-1.9	-2.2	at 25	0.23	0.32	4.12	13.35
6835	June 30	12.60	.16	2.23	-1.3	-1.5	at 27	.16	.22	4.00	29.63
a 6855	July 13	13.1661	-3.2	-3.8	at 23.5	.46	.41	6.40	24.41
6893	July 28	14.30	.12	2.42	-2.9	-3.74	at 32	.67	.77	5.53	6.34
6925	Aug. 18	14.07	.12	1.69	1.09	5.53	6.68
7260	Sept. 24	13.88	.09	1.19	1.93	5.44	7.47
7287	Oct. 15	14.40	.10	.61	2.35	6.37	8.84
7296	Oct. 23	14.2250	2.90	6.58	9.63
7305	Oct. 30	14.22	.12	.28	-3.2	-7.1	at 24.1	3.29	2.97	6.74	9.87
7310	Nov. 5	13.93	.09	None.	-4.1	-8.2	at 23.7	3.14	2.51	6.72	9.36

a All figures in italics give analyses of apples held in the ice box since the preceding date.

DISCUSSION OF CHANGES OCCURRING DURING GROWTH.

In terms of total solids the composition of the summer apples is shown graphically in figures 13 to 15, inclusive. The results of this work give a much more complete account of the life history of the apple than was obtained in the previous year. By referring to the graphic chart for the Early Strawberry (fig. 13) as an illustration of the summer apples, it is seen that the starch content increased from June 11, 1903, the date of the first examination, until June 25, when the maximum starch content was attained. From that time until August 5 the starch steadily decreased, and on that date the sample examined still contained 6 per cent of starch based on the total solid content of the apple.

The apples were not in a good state of preservation, however, for by this time all had fallen from the trees and none in a condition suit-

able for analysis could be obtained. The last stages of ripening, therefore, could not be followed. This same observation is true of the other varieties of summer apples. No sample could be obtained which became fully ripe in the chemical sense—that is, in which the starch had completely disappeared.

In the first sample of summer apples examined very little sucrose

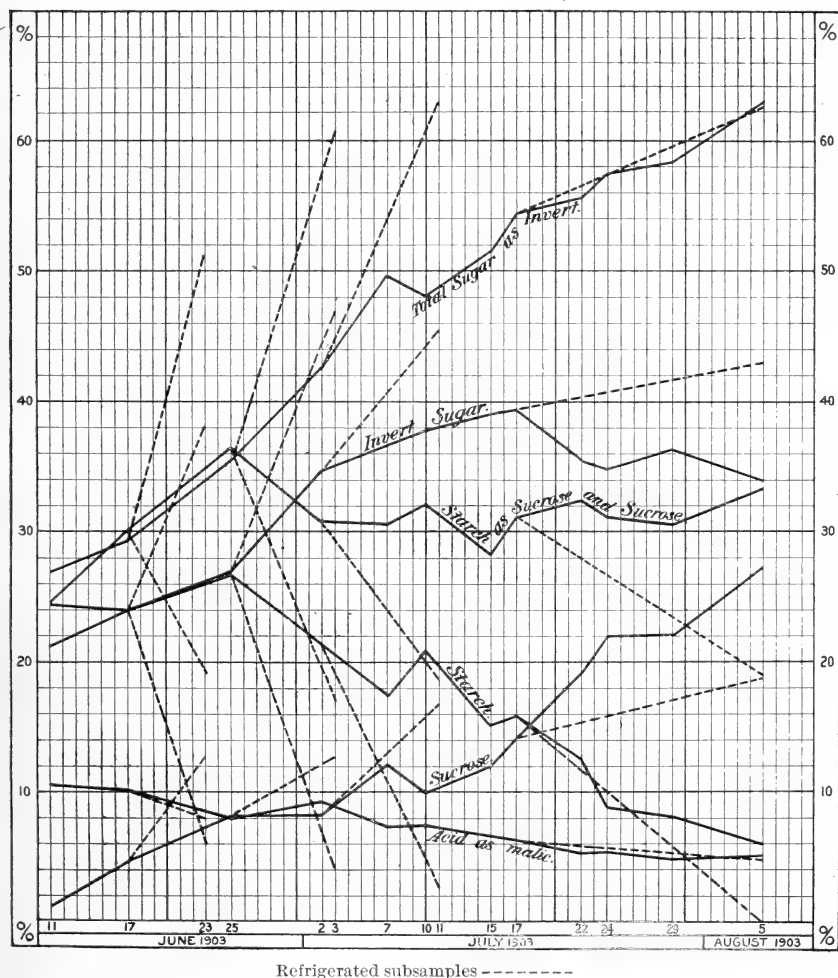


FIG. 13.—Chart showing chemical changes in summer apples (Early Strawberry) during growth (total solids basis).

was found, showing that the work was begun early in the life history of the fruit. The content of sucrose increased steadily, however, until the end of the experiment. It is specially noteworthy that the percentage of sucrose increased even while the starch was forming. At the beginning of this work the sample contained a larger percentage

of invert sugar than starch and the percentage of invert sugar increased steadily until the sample was examined on July 4. Later the percentage of invert sugar decreased. This does not mean, however, that an actual loss of invert sugar is indicated, as will be seen by consulting figure 21, in which the composition of the fruit in grams

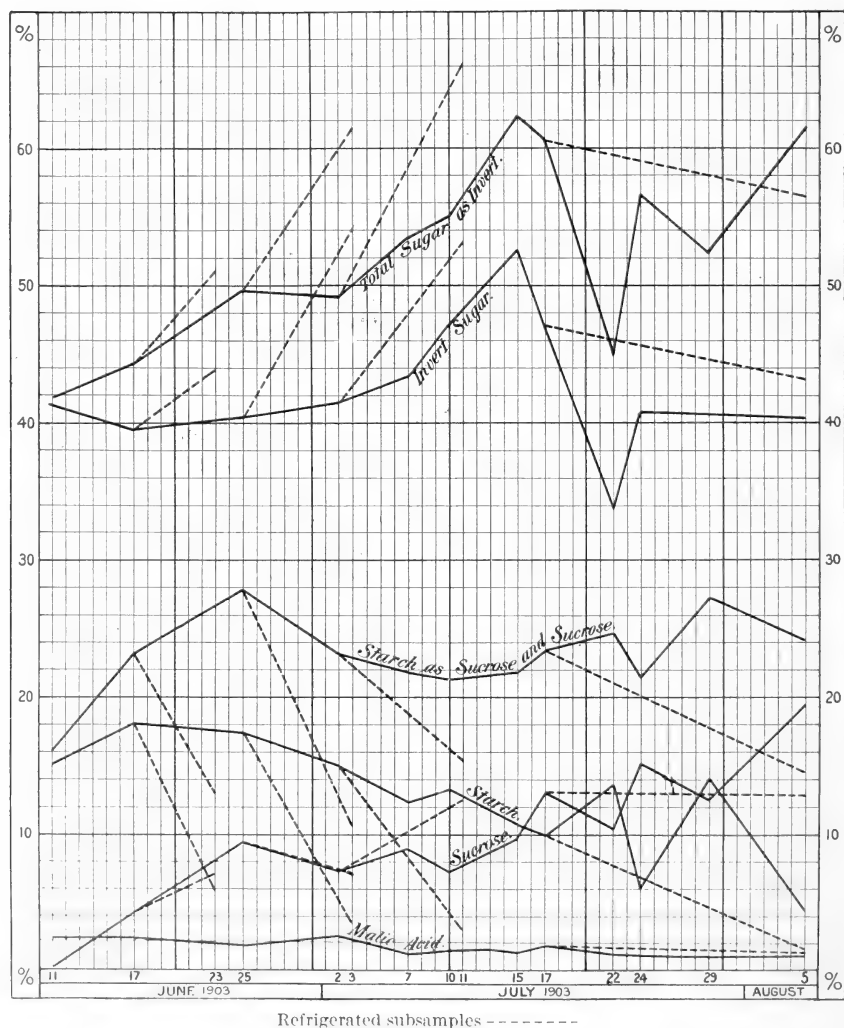


FIG. 14.—Chart showing chemical changes in summer apples (Bough) during growth (total solids basis).

per apple is given. The reason for the apparent loss of invert sugar—that is, for the decrease in the percentage of invert sugar—is due to the rapid growth of the apples after the date mentioned. This growth of the apples after July 2 amounted, in the case of the Early Strawberry apple, to 29 grams, the weight increasing from 43 grams on July 2 to 72 grams on August 5.

In figure 16 the average composition of the three varieties of summer apples during growth is shown graphically in the form of composite curves. In this it is seen that the conclusions already drawn from the Early Strawberry apple were true of each of the varieties. The curves representing the content of each of the substances deter-

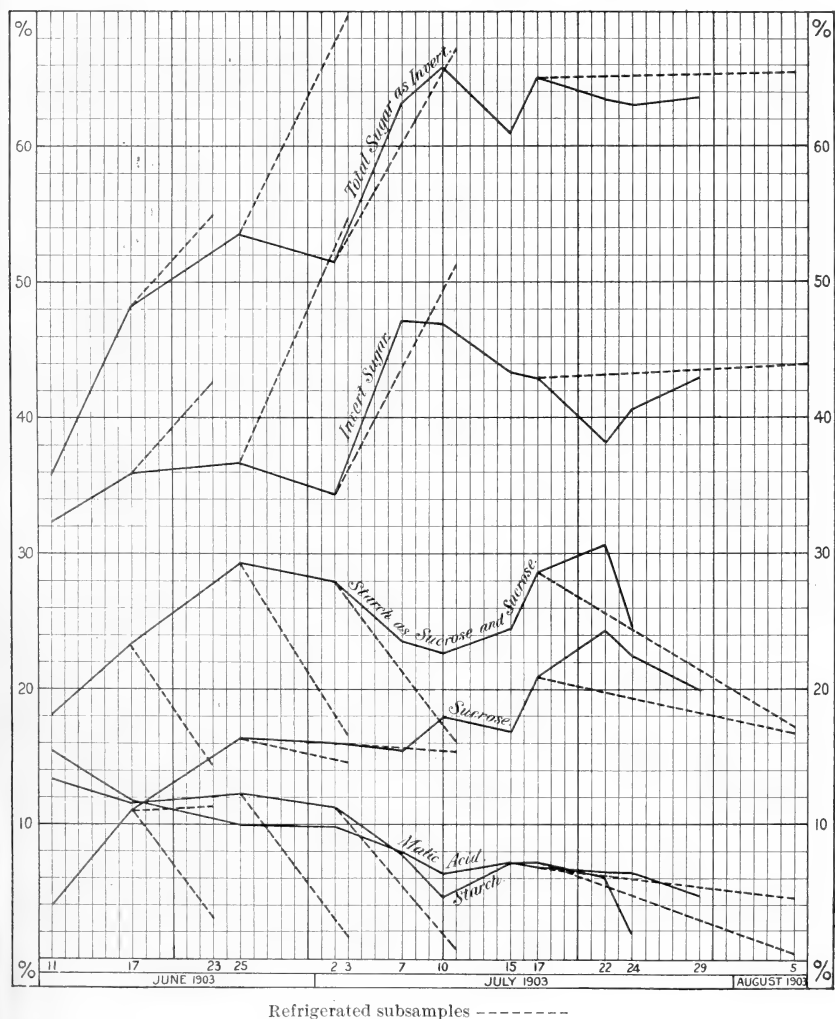


FIG. 15.—Chart showing chemical changes in summer apples (Yellow Transparent) during growth (total solids basis).

mined from time to time in the summer apples are very irregular. This is apparently due to the fact that summer apples usually do not ripen with the uniformity of later varieties. An effort was made at the beginning to secure apples of uniform ripeness, but in spite of

this it doubtless happened that in some cases a larger percentage of relatively green apples was obtained than in others. In addition to this, the chemical composition of the apples changes very rapidly after they are picked from the trees. This is a matter which was not well understood at the beginning of the work and the error caused

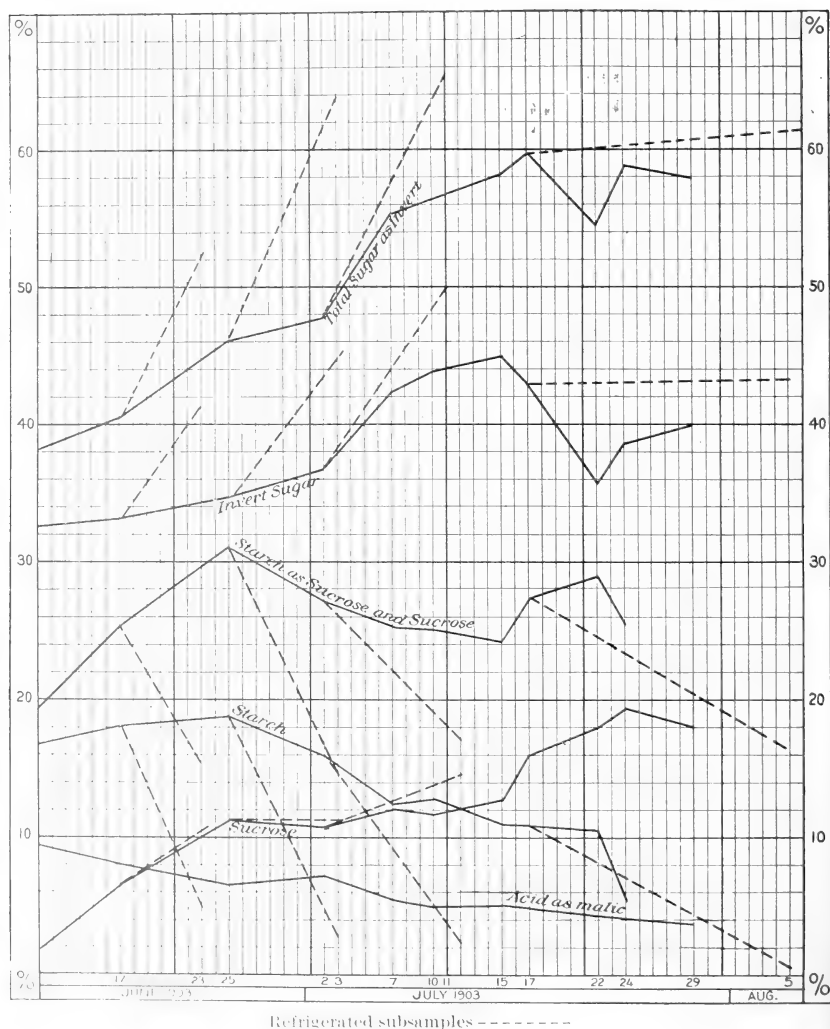


FIG. 16.—Chart showing average chemical changes during growth in the three summer varieties (total solids basis).

thereby was not guarded against as fully as it might have been. Owing to the fact that the apples were grown in West Virginia, from twenty four to thirty six hours elapsed from the time they were picked until analysis was begun in the laboratory. It was supposed that this lapse of time would not cause great changes in the composition of the

apples, but it was found that such was not the case. At each picking the sample was divided into two portions, one of which was placed in a refrigerator at a temperature of from 12° to 15° C. (53.6° to 59° F.), and the apples so kept were examined after the lapse of several days. The results of the examinations of these subsamples which were kept in the refrigerator are printed in italics in Table VI, and they are also expressed on the charts (figs. 13 to 16) by dotted lines.

Referring to figure 13, in which the composition of the Early Strawberry apples, based on total solids, is shown graphically, it is seen that the composition of the sample of apples received June 17, 1903, changed with remarkable rapidity. The sample at that time contained 24 per cent of starch, 4.6 per cent of sucrose, and 24 per cent of invert sugar. After being kept in the refrigerator six days, it was examined and found to contain 5.9 per cent of starch, 12.9 per cent of sucrose, and 38.2 per cent of invert sugar. Thus, in six days the apples which were picked from the trees and kept in the dark at a temperature considerably lower than that to which those remaining on the trees were exposed, contained less starch than the apples which ripened fully on the trees forty-three days later, and almost as high a content of invert sugar. At the same time it must be borne in mind that the apples remaining on the trees during this period continued to grow the whole time, whereas the transformation of starch in case of the apples stored in the ice box was limited to a few days. For this reason the parallel drawn is not entirely applicable.

On several succeeding dates the samples drawn from the trees were preserved in the refrigerator for a few days with similar results. It was found with each successive picking that apples which were stored in the refrigerator developed somewhat more slowly than on the preceding occasion. Thus, each succeeding curve representing the change of the apples kept in the refrigerator is a little less vertical than that preceding it. This demonstrates that the less mature the fruit is when gathered the more rapid are the changes tending to maturity after picking. It would seem, therefore, that, from a commercial standpoint, apples which are fairly mature may be expected to retain a more constant composition than those picked in an immature state. The same generalization also applies to the charts representing the changes in composition in other varieties of summer apples, and are especially borne out in the composite chart (fig. 16), which gives the average of the results obtained with the three varieties of summer apples.

The work on the winter apples was much more satisfactory than that on the summer apples, because of the fact that they ripened more simultaneously and the problem of securing a representative sample was not so difficult. For this reason the curves representing the changes of composition of the winter apples (as shown by figures 17

to 20, inclusive) are much more uniform than in the charts just preceding, which represent the composition of the summer apples. The work on the winter apples began on June 16, 1903, and extended until November 5, 1903. These curves illustrate much better the early life history of the fruit than those representing the work of the previous year. As in that year, the sucrose curve is almost exactly the reverse of the starch curve. This is only true, however, after the maximum content of starch has been reached, which was between June 30 and July 28 with the Ben Davis apples, on July 28 with the Huntsman apples, and on June 30 with the Winter Paradise apples. It must be understood that no one of these dates is suggested as the exact date of the maximum content of starch in the apple. It is only intended to represent the maximum content of starch on the various dates when the apples were examined. On the whole, however, the maximum content can not have varied greatly from the date given, and the maximum percentage determined must also be approximately correct.

The observation as to the uniformity of results applies equally to all of the determinations made. On the date of the first examination—June 16—the content of sucrose based on total solids was 4 per cent. The percentage of sucrose increased regularly until the last examination, which was made on November 5, when it amounted to 25.4 per cent of the total solid content of the apple, the rate of increase being apparently no greater before the maximum content of the starch than afterwards. It would appear that during its own growth and accumulation a portion of the starch is converted into sucrose. Unlike the summer apples, the percentage of invert sugar here increased from the date of the first examination to approximately the date of the last, so that even in percentage composition the amount of invert sugar present did not reach its maximum until the maturity of the fruit.

In all three of the varieties of winter apples studied the percentage of malic acid decreased from the first examination to the full maturity of the fruit. The percentage of total sugar estimated as invert sugar increased steadily from the first examination to full maturity. It is a notable fact that after the maximum content of starch is reached the percentage of starch and invert sugar taken together remains approximately constant. As in the case of the preceding studies, the average composition of the three varieties of winter apples has been expressed in the form of a composite chart which is given in figure 20.

In many respects it was considered that a graphic statement showing the actual increase in weight of the various constituents of the apple determined would have a more definite meaning than the changes in chemical composition on the percentage basis. Before examination each sample was weighed so that the data were secured for this calculation. In figures 21 to 28 the changes of the apples just considered, both summer and winter varieties, are represented in terms of grams per apple.

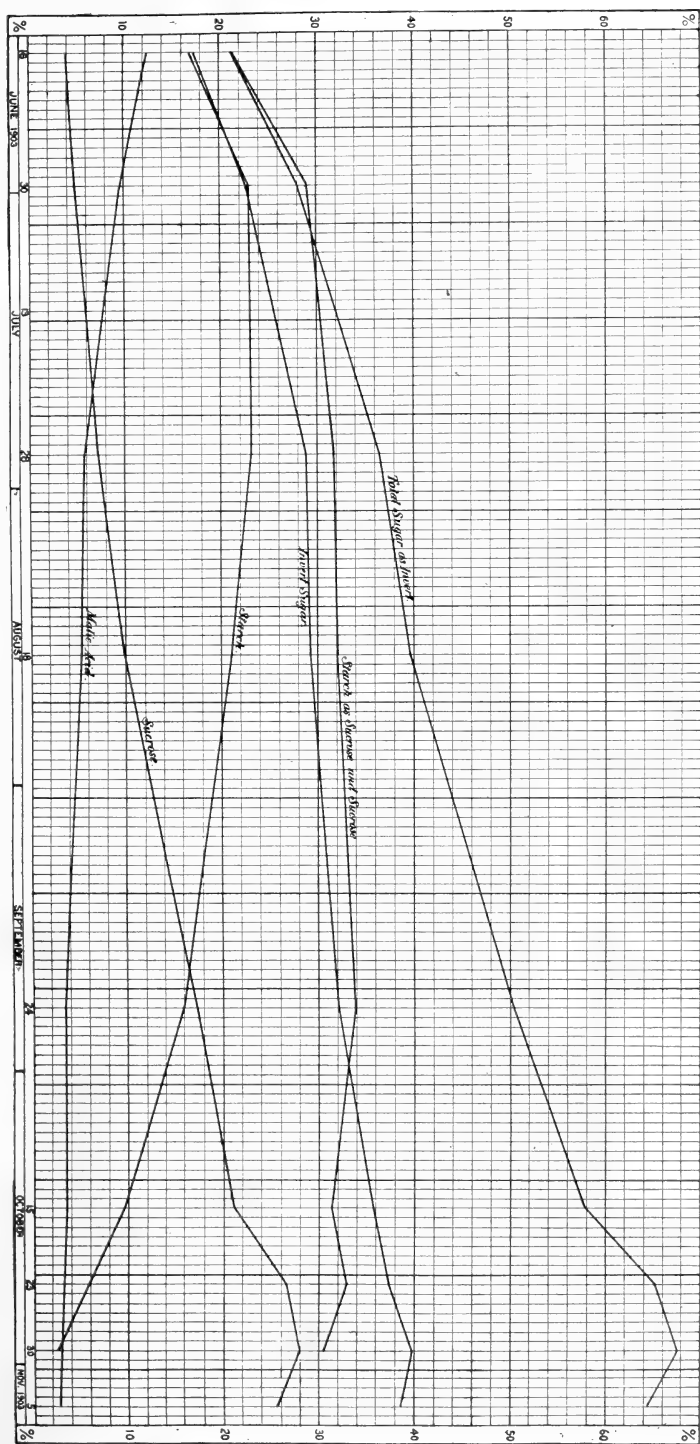


FIG. 17.—Chart showing chemical changes in winter apples (Ben Davis) during growth (total solids basis).

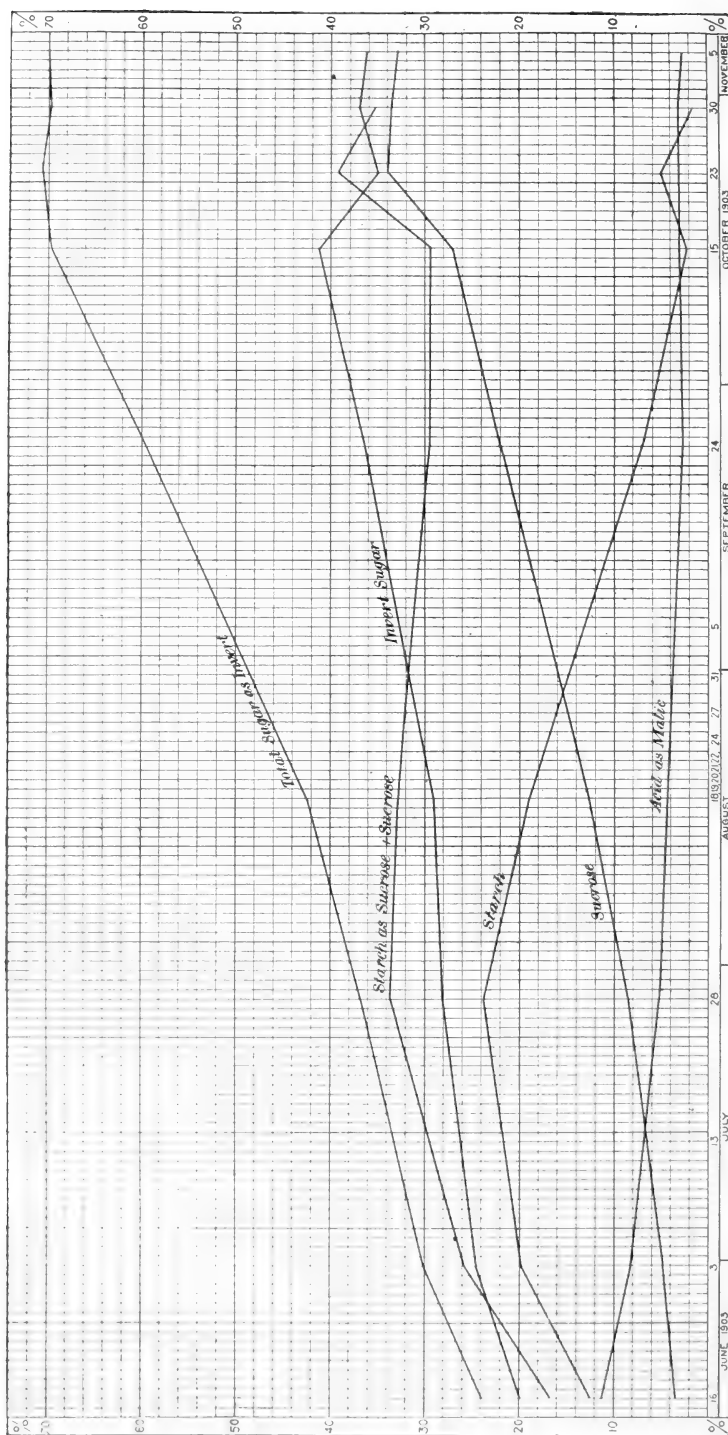


FIG. 18.—Chart showing chemical changes in winter apples (Huntsman) during growth (total solids basis).

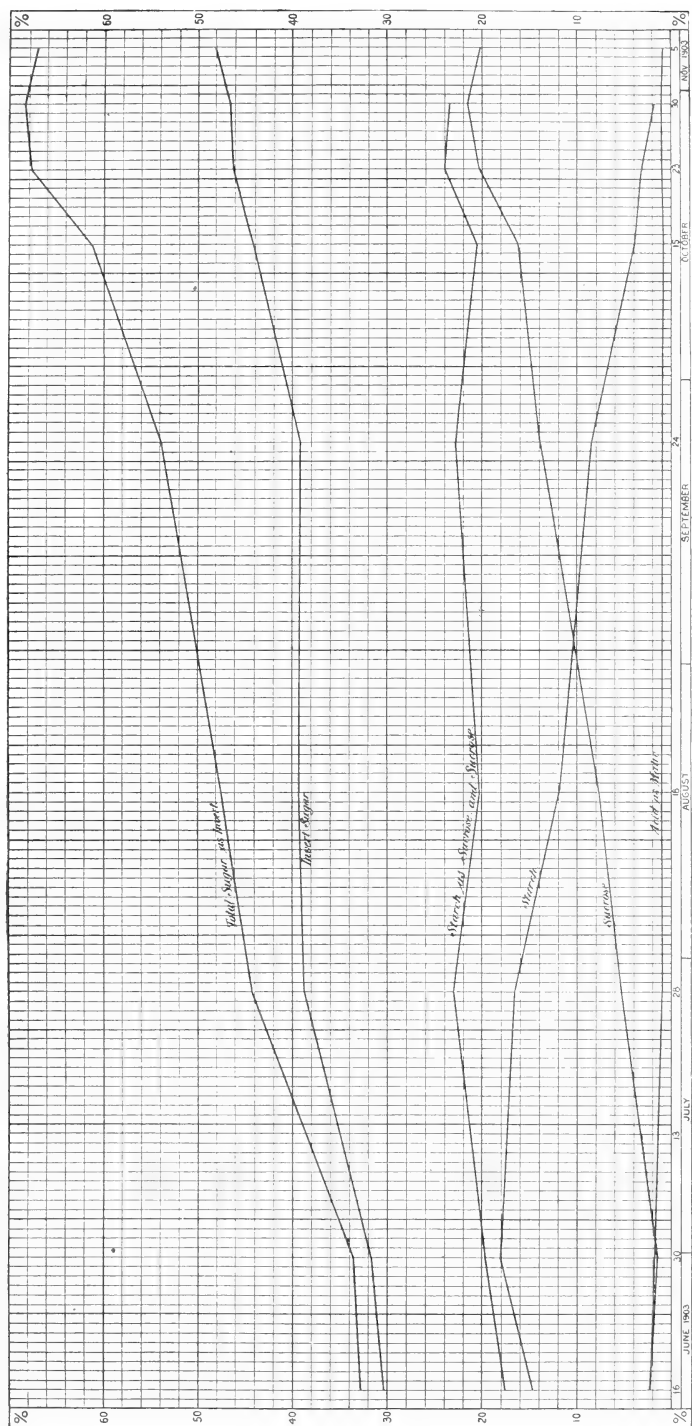


FIG. 19.—Chart showing chemical changes in winter apples (Winter Paradise) during growth (total solids basis).

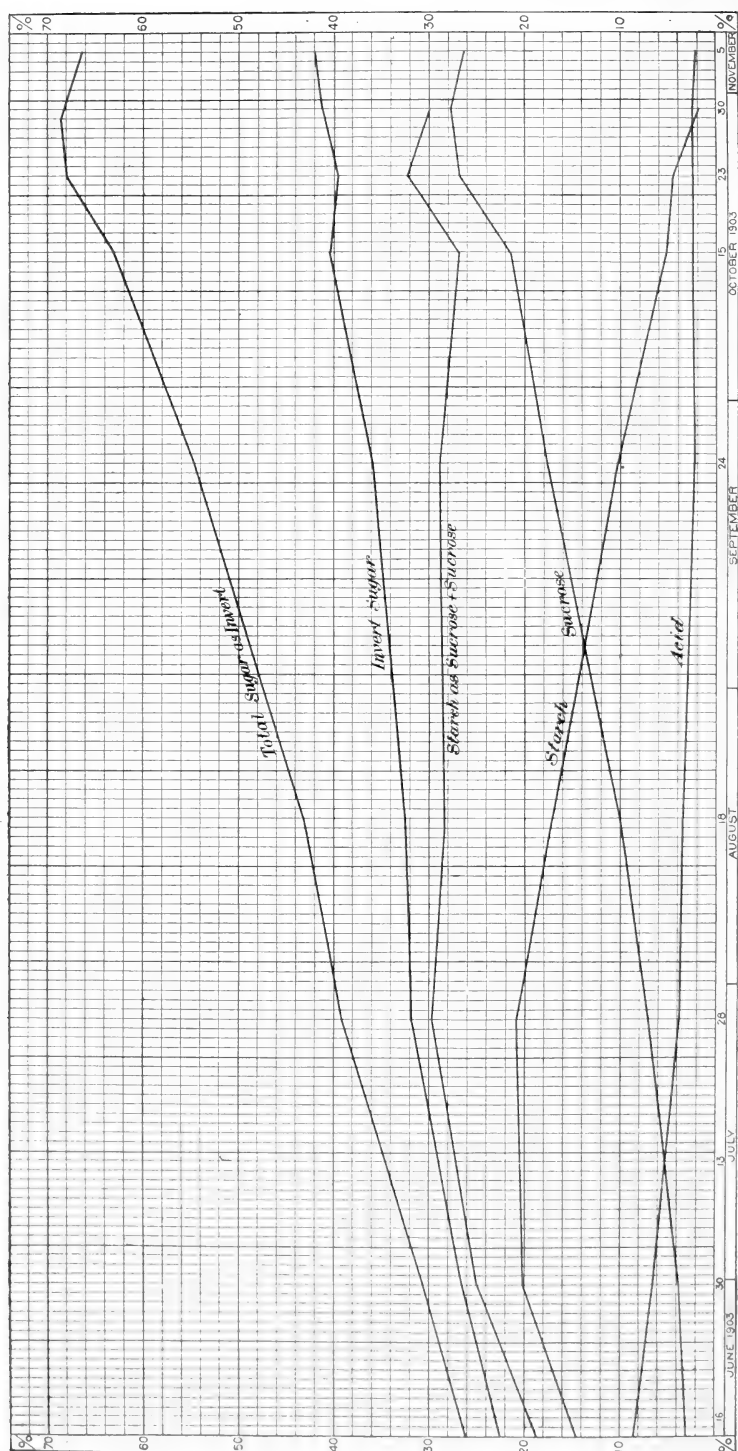


FIG. 20.—Chart showing average chemical changes during growth of the three winter varieties (total solids basis).

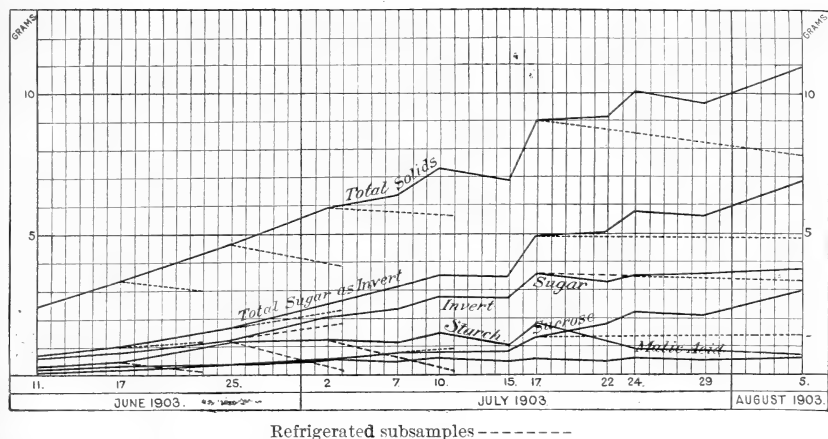


FIG. 21.—Chart showing chemical changes during growth of summer apples (Early Strawberry)—in grams per apple.

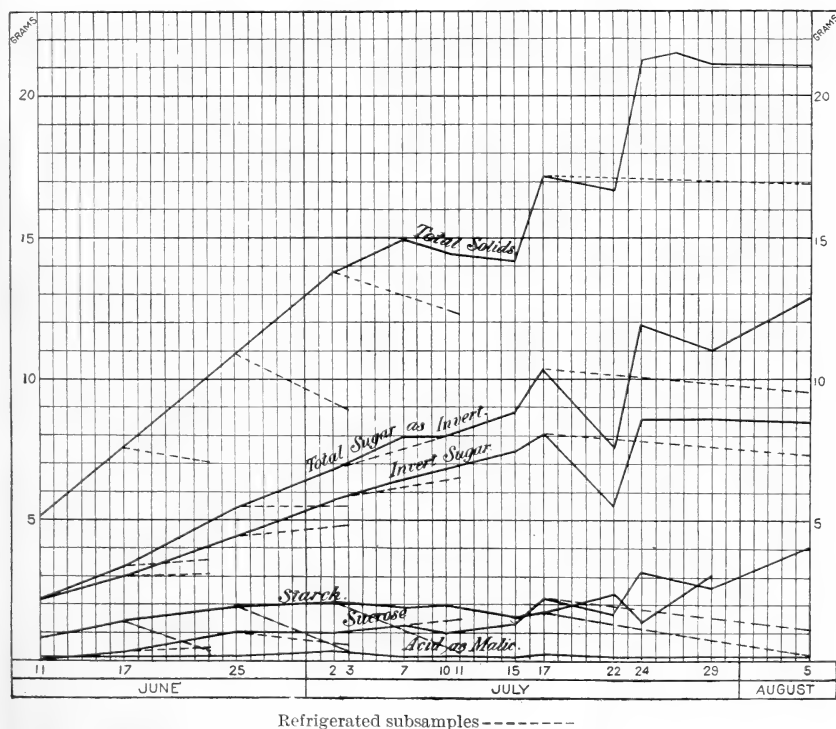
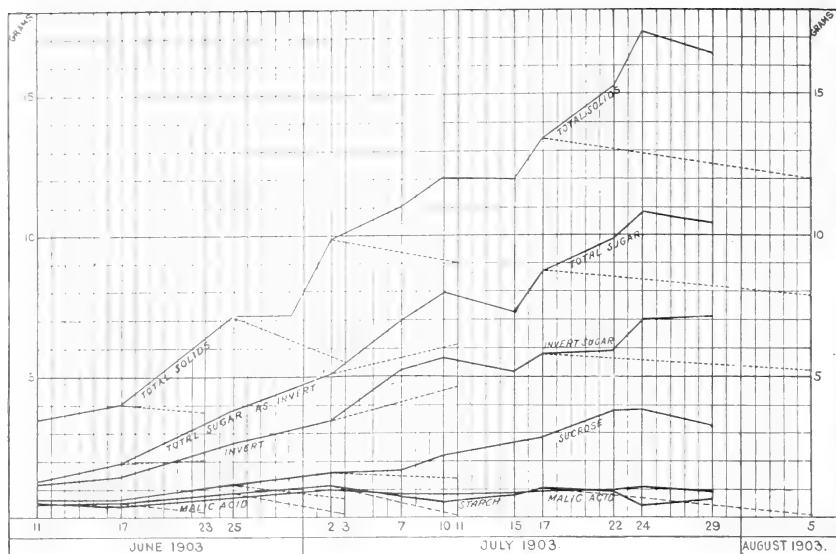
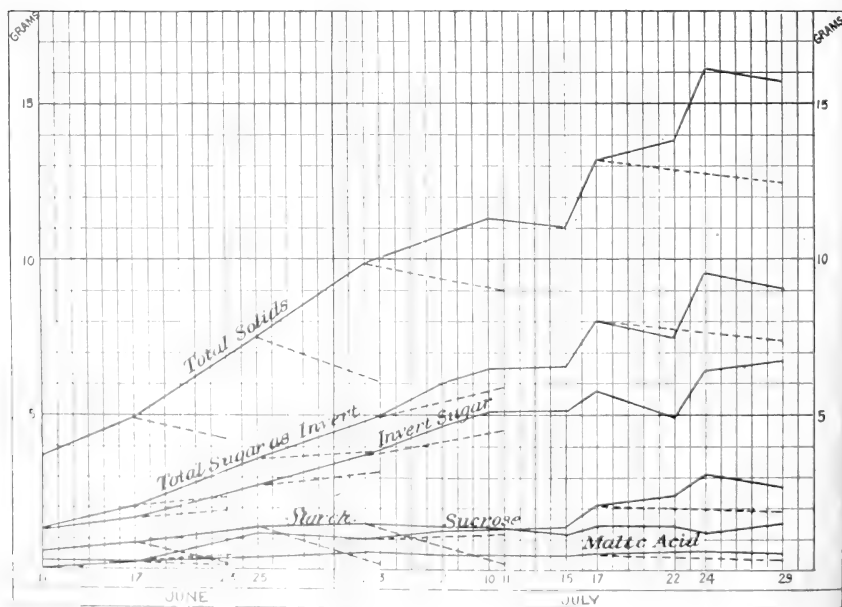


FIG. 22.—Chart showing chemical changes during growth of summer apples (Bough)—in grams per apple.



Refrigerated subsamples - - - - -

FIG. 23. -Chart showing chemical changes during growth of summer apples (Yellow Transparent)—in grams per apple.



Refrigerated sub-samples - - - - -

FIG. 24. -Chart showing average chemical changes during growth of the three varieties of summer apples—in grams per apple.

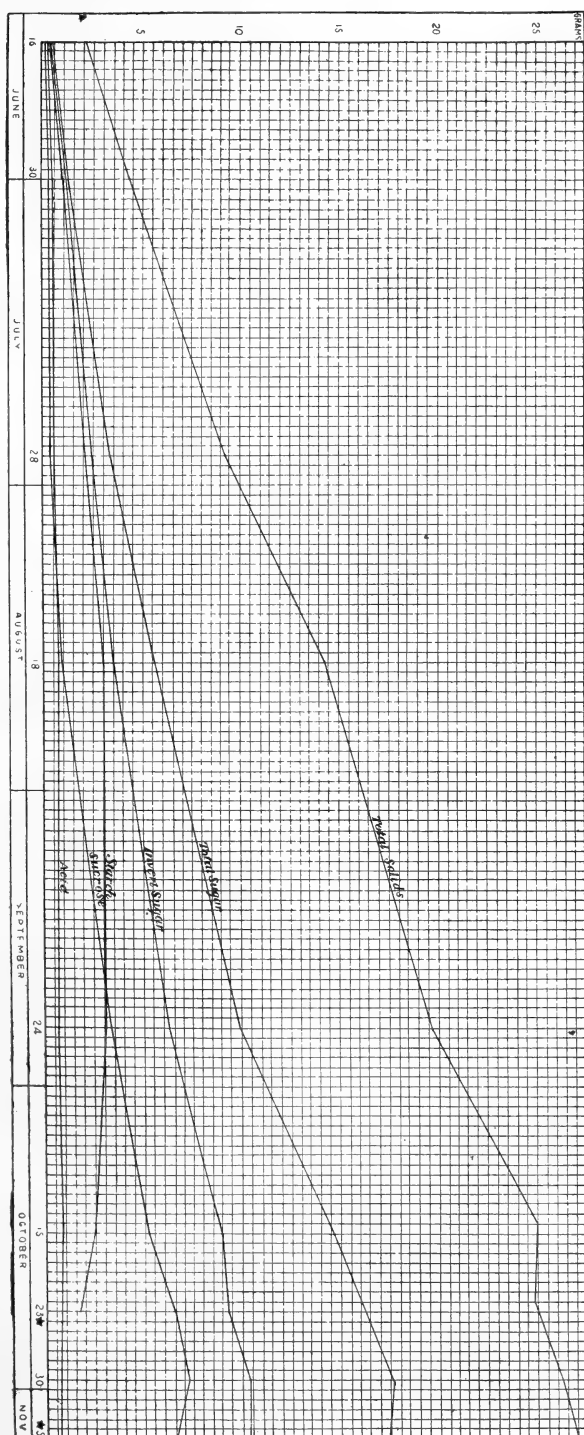


FIG. 25.—Chart showing chemical changes during growth of winter apples (Ben Davis)—in grams per apple.

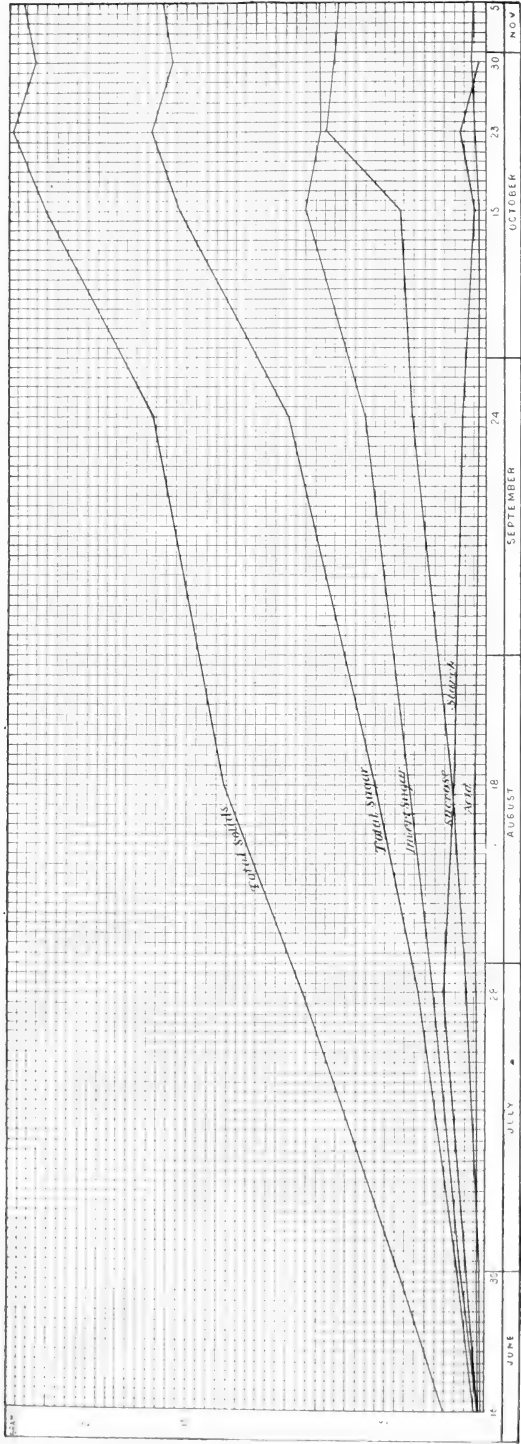


FIG. 26.—Chart showing chemical changes during growth of winter apples (Huntsman)—in grams per apple.

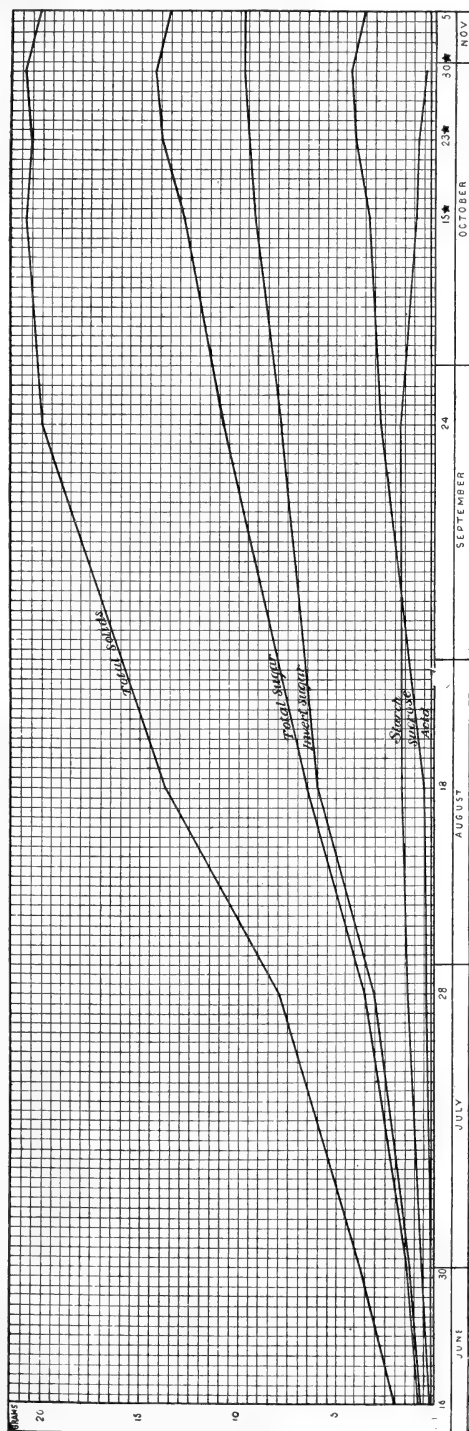


FIG. 27.—Chart showing chemical changes during growth of winter apples (Winter Paradise)—in grams per apple.

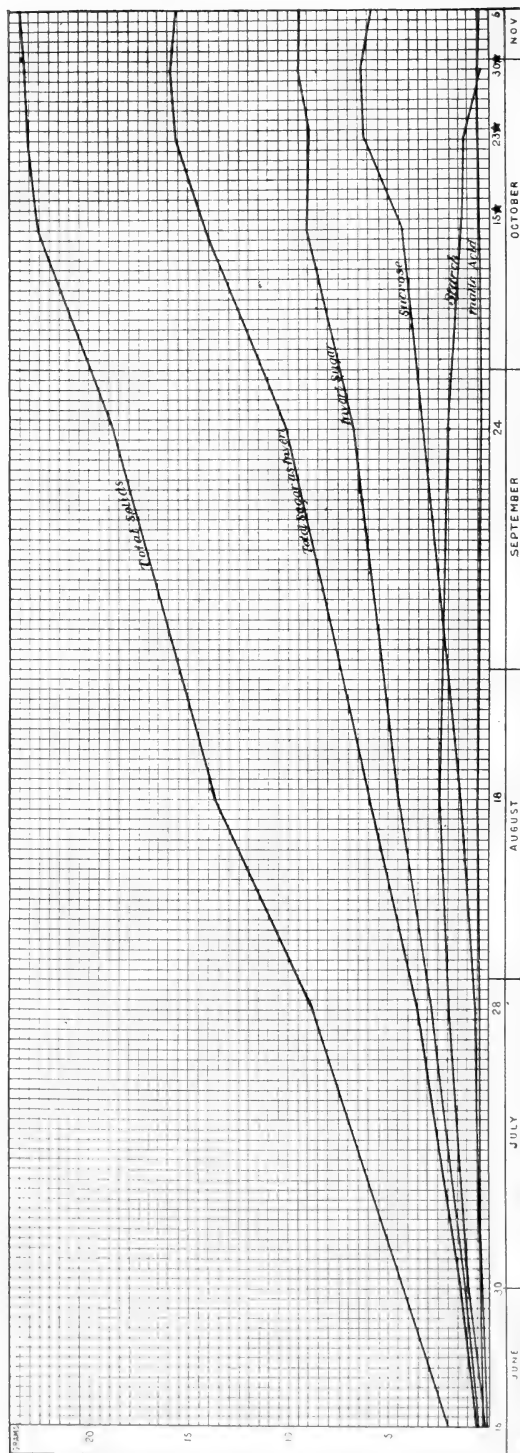


FIG. 28.—Chart showing average chemical changes in the three varieties of winter apples examined—in grams per apple.

In the case of the summer apples the curves representing the changes of composition in terms of grams per apple, as in the preceding charts in which percentage composition alone was represented, are very irregular, owing to the fact, as previously stated, that it was very difficult to secure a representative sample, the season of ripening being so irregular that it was impossible to secure samples having an equal number of relatively ripe apples. Irregularities of the curves in figures 21 to 24, representing the summer apples, are due to this fact.

In the charts just mentioned the actual weight of starch increased from the date of the first analysis until July 17 in the case of the Early

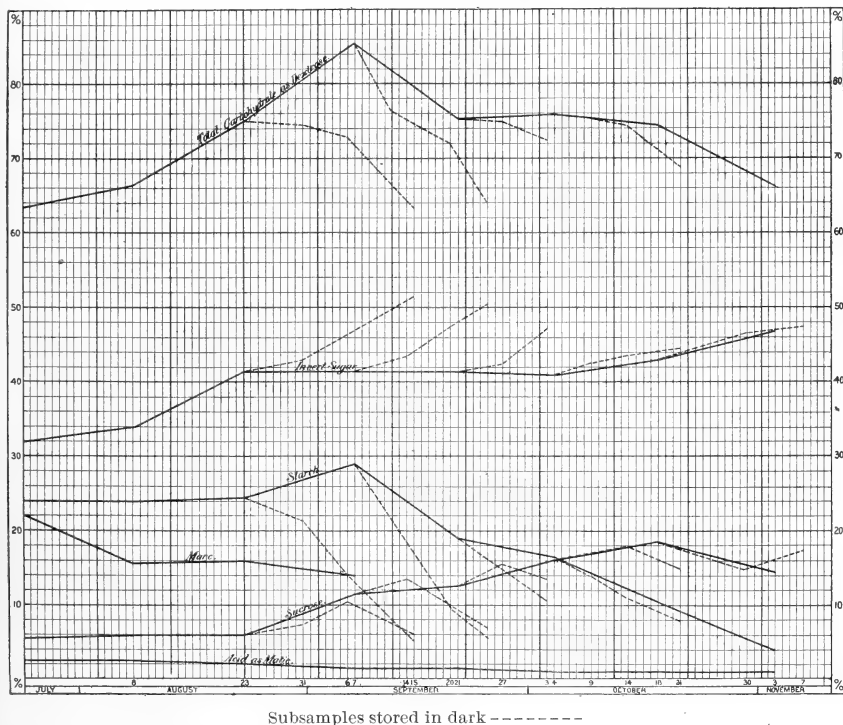


FIG. 29.—Chart showing results of analyses of growing apples by Lindet (calculated to an assumed basis of 20 per cent of total solids).

Strawberry apples, and until July 2 in the case of the Yellow Transparent. The percentage of starch in the Yellow Transparent apples remained almost constant until July 23. The irregularities in the curves representing the starch content in the Bough apples were so great as to render conclusions from this curve impracticable. It is probable that the maximum starch content of this variety was attained on July 2, the same date as in the case of the Yellow Transparent. On two subsequent occasions, however (July 22 and 29), a higher content of starch was noted, but on each of these dates the content of sucrose

was correspondingly lower than would have been expected from preceding and succeeding analyses. On account of the irregularities in the starch content of the Bough apples, the last of the curve representing the starch content of the composite sample is of little value, and the same is true of the curve representing sucrose. On the whole it is apparent that the starch content increased until the early part of July, after which it steadily decreased. On the other hand, the sucrose content increased regularly from the first analysis to the full maturity of the fruit. The same is true of the invert sugar.

Turning now to the succeeding charts (figs. 25 to 28) the curves representing the changes of composition of the winter apples are again found to be more satisfactory than in the case of the summer apples. Owing to the more uniform ripening of this fruit and the consequent greater uniformity attained in the selection of samples, the curves are much more regular. The maximum content of starch in winter apples was attained on August 18, at least a month later than in the case of the summer apples just considered. After reaching the maximum starch content the sucrose increased more rapidly than before and maintained a fairly rapid increase until the apples were fully matured. The content of invert sugar, total sugar, total carbohydrate, and total solids remained reasonably constant from the first analysis to the full maturity of the fruit.

In the series of plattings indicated by the star after the date of analysis (figs. 25, 27, and 28), the analyses were calculated to the weight of the largest average weight of apples received up to the time when analyzed, as the sample received consisted of smaller apples.

As in the case of the summer apples duplicate samples were taken, one of which was subjected to analysis immediately on its receipt in the laboratory, the other being placed in a refrigerator and allowed to stand for some time before it was examined. Owing to the press of other work, however, examinations of these subsamples were not made.

In figure 29 are given representative results obtained by Lindet which illustrate somewhat more clearly the change of composition of apples during ripening than does the tabular form in which they were given by the author. The apples were of cider varieties. The results as given by him were entirely on the original composition of the apples. The content of water was not given, and for the calculation of the figures here given a total solid content of 20 per cent was assumed. While this is probably not entirely correct, it can not be greatly different from the true amount. One interesting feature of Lindet's work was the storage in the dark of a portion of the samples analyzed at each picking. The temperature at which the apples were stored is not given. Lindet did not appear to observe the more rapid ripening of the immature fruit, and that fact was not apparent in the table of composition which he published. The graphic representation here

given, constructed from his figures, makes it evident. This article was found subsequent to the completion of the writers' work on the same subject, but it is confirmed by the latter in all respects.

As indicated by the results of Lindet and those obtained by the writers (as given in figures 13 to 16), the green apples ordinarily mature much more rapidly when stored than when left on the tree. It is also equally true that the apples which were picked earliest matured more rapidly than those picked at a later date, and the invert sugar content of the earliest picked apples reached a higher figure after picking than that of those picked at a later time. "Maturing" in this connection is regarded from a chemical point of view only, especially with regard to the decrease of malic acid and starch, and the increase of sucrose and invert sugar.

Germination tests, conducted at 20° C. on the seeds of all samples of the six varieties of growing apples studied in 1903, were made by the seed laboratory of the Bureau of Plant Industry, Department of Agriculture, under the direction of Dr. J. W. T. Duvel. In all cases negative results were obtained, except in two instances—Huntsman (serial No. 7286), in which case a germination of 2 per cent was noted, and Winter Paradise (serial No. 7260), which gave 6 per cent.

METHODS OF ANALYSIS.

PREPARATION OF SAMPLE.

The sample was quartered, cores removed, twice passed through a meat grinder, and received in a fruit jar (provided with a cover) from which the various portions were weighed out.

The methods of the Association of Official Agricultural Chemists were followed in general, but on account of various changes introduced as the work progressed, they are given here in detail.

DETERMINATION OF TOTAL SOLIDS.

A sample of about 10 grams was weighed into a tared flat-bottomed lead dish, stirred with a little water, and evaporated to nearly constant weight in a vacuum oven at a temperature not higher than 70° C., and in a vacuum of 15 to 20 inches.

DETERMINATION OF TOTAL ACID.

Samples weighing 10 or 20 grams were weighed in a counterpoised sugar dish, and washed into a beaker of about 400 cc capacity with about 300 cc of water, brought to a boil, cooled somewhat (while the beaker remained covered with a watch glass), titrated with tenth normal sodium hydroxid, using phenolphthalein as indicator, and the result expressed as malic acid.

DETERMINATION OF SUGARS.

Five times the normal weight for the Schmidt and Haensch polariscope, 130.24 grams, was weighed in a counterpoised sugar dish, washed into a 500-cc flask, and 25 cc of basic lead acetate^a was added, the mixture made nearly up to the mark,

^a Prepared according to the directions given in Bul. 65, p. 84, Bureau of Chemistry, U. S. Dept. of Agr.

shaken, and allowed to stand over night. The solution was then filtered, the lead nearly all removed by dry sodium sulphate, followed by dry sodium carbonate, the solution again filtered, and the filtrate used in the determination of sugars by polarimetric and gravimetric methods. The Clerget method was used for the polarimetric work, calculating by the formula as modified by Tolman,^a viz:

$$s = \frac{a - b}{141.85 + .062b - \frac{T}{2}}$$

Soxhlet's method was used in the determination of reducing sugar as invert before and after inversion. The tables of Meissl and Wein were used, and the cuprous oxid was filtered off on Gooch crucibles and weighed as such, as described by Munson.^b

DETERMINATION OF STARCH.

Fifty grams of pulp were weighed into a clean cloth bag, the mouth of the bag closed with a rubber band, and the contents squeezed with the hand or lemon squeezer, and then washed with portions of about 25 cc of water until the washings amounted to about 250 cc. The last washings were in all cases neutral to litmus and free from reducing sugar. The starch was settled from the washings by means of a centrifugal machine, repeatedly washed by stirring with fresh portions of water and settling, and transferred to a 300-cc flask, roughly graduated at 200 cc. The marc from the cloth bag, separated as completely as possible by scraping with a spatula, was washed into the flask, and the whole made up to 200 cc. Twenty cubic centimeters of approximately 25 per cent hydrochloric acid (sp. gr. 1.125) was added, and the whole heated for three hours in, not on, the steam bath under an air condenser. The mixture was then cooled, almost neutralized by sodium hydroxid, cooled again, made up to 300 cc, filtered, and 25 cc portions employed for the determination of dextrose by Allihn's method.^c

DISCUSSION OF METHOD FOR STARCH.

Determinations by the above method are all 0.5 to 0.8 per cent too high, owing to bodies not starch which remain in the marc and become partially hydrolyzed to reducing bodies by the acid treatment.

The supernatant liquors from the centrifugal machine always give a test for starch, but the quantity of starch present is very minute.

Satisfactory results could not be obtained by the diastase method,^d because it was impossible to break all the cells. The diastase did not have ready access to the swollen starch grains, and the complete washing out of dextrin was not possible. The direct extraction of the sugars by alcohol was tried, but was found less convenient than the water extraction.

Other methods for the determination of starch in fruits have been described, among which the methods of Browne and Lindet are given as worthy of special consideration.

^a Bul. 73, p. 70, Bureau of Chemistry, U. S. Dept. of Agr.

^b Ibid, p. 65.

^c Bul. 65, p. 49, Bureau of Chemistry, U. S. Dept. of Agr.

^d Ibid, p. 58.

C. A. Browne, jr.,^a determined starch by the following method: One hundred grams of finely grated pulp were washed upon a muslin filter with repeated quantities of cold water until the filtrate amounted to two liters, the muslin being squeezed after each addition of water. The washed out starch was allowed to settle, the supernatant liquid poured off, and the starch collected on a hardened filter and washed with water. The starch was determined by the official diastase method with the exception that sodium hydroxid instead of sodium carbonate was used for neutralization after the hydrolysis with hydrochloric acid. The residue on the filter was also run by the diastase method, in case it showed any reaction with iodine. Not more than 0.1 to 0.2 per cent of starch, calculated on the original bulk, was ever found in this residue.

Lindet^b used the following procedure, depending on the solvent action of salicylic acid on starch: One hundred grains of pulp were placed on a filter and washed with 5 to 6 liters of water, to which a little mustard oil was added to prevent fermentation. The filter and contents were introduced into a flask with 250 cc of water, 2 grams of salicylic acid, and 100 grams of salt, and boiled for three hours under a return condenser. The volume of the resulting solution was measured and the solution polarized, corrections being made for the volume of solid constituents. The percentage of starch was calculated from the rotation observed, the rotatory power of the dextrin being taken as $(\alpha)_D = +177$. The method was checked by hydrolyzing the dextrin obtained to dextrose and determining the latter by means of Fehling's solution.

II. INSOLUBLE CARBOHYDRATES OR MARC.

PECTIN BODIES.

HISTORICAL REVIEW.

Good reviews of the literature and descriptions of pectins have been made by von Lipmann,^c Tollens,^d Hebert,^e and by Mangin,^f the latter having taken up the work from the botanist's point of view. In order to bring the subject before American investigators the present review is presented.

The pioneer work in the field was done by Braconnot,^g who found pectins very widely distributed in plants, occurring in the dahlia, in Jerusalem artichokes, celery, carrots, onions, in stems and leaves of

^a J. Am. Chem. Soc., 1901, **23**: 869.

^b Ann. agron., 1894, **20**: 5.

^c Chemie der Zuckerarten, 1895, p. 924-936.

^d Handbuch der Kohlenhydrate, 1888 ed., p. 242-246, 1895 ed., p. 242-247.

^e Ann. agron., 1900, **26**: 34-50.

^f J. bot., 1891, **5**: 400, 440; 1892, **6**: 12.

^g Ann. chim. phys., 1825 [2], **28**: 173.

herbaceous plants, in the cortical layers of trees, and in fruits. So constant was its occurrence in his researches that this author regarded it as one of the principal constituents of all plants. He considered it to be similar, if not entirely identical, with the principle in plants vaguely described as jelly. The method given by Braconnot for obtaining pectin (as pectic acid) from roots containing starch is to pulp the roots, wash out with water to remove sugar, then boil the marc with dilute hydrochloric acid, wash, and heat the starch-free and sugar-free marc with very dilute potash or soda. There results a mucilaginous slightly alkaline liquid, from which hydrochloric acid separates pectic acid as a jelly. So prepared, pectic acid had a feebly acid reaction to litmus and was slightly soluble in hot water, but the filtered liquor did not precipitate on cooling, and barely reddened litmus. It was, however, coagulated by alcohol, by metallic salts, and even by sugar. Dried on a capsule it appeared as transparent leaflets which loosened from the capsule as they dried. These were slightly swelled by cold water, and dissolved slightly in boiling water. A potassium salt was separated by precipitating its water solution with alcohol, excess of alcohol extracting coloring matter and excess of alkali. This salt was very soluble in water, possessed a flat insipid taste, and yielded 15 per cent of potassium, calculated from the sulphated ash. Its water solution was coagulated by salts, and by alcohol and sugar, and gave precipitates with solutions of salts of heavy metals. An ammonium salt was prepared which possessed properties similar to those of the potassium salt. The use of these salts for the preparation of jellies was suggested, and experiments are described in which beautiful jellies were obtained.

The pectic acid was attacked only slightly by concentrated sulphuric acid in the cold. Nitric acid yielded oxalic acid and a white powder which was treated with ammonia to separate from calcium oxalate. The ammonia solution, acidulated, gave a granular crystalline substance which he believed to be mucic acid. Braconnot concluded the article by proposing for this acid the name pectin, from the Greek word *πηκτις*, meaning coagulum.

Vauquelin^a worked on the carrot, obtaining pectin from the juice by boiling in order to clarify, then precipitating with alcohol; and from the marc (as pectic acid) by boiling with dilute caustic potash and precipitating with calcium chlorid, or better, barium hydroxid, filtering, treating the filtrate with sulphuric acid, and then with potassium hydroxid, finally precipitating with hydrochloric acid. Distilled or filtered rain water is stated to be necessary.

Braconnot^b in 1833 described the pectin separated from oak bark by solution in alkali. It was not precipitated from solution in alkali by

^a Ann. chim. phys., 1829 [2], 41: 46.

^b Ann. Chem. (Liebig), 1833, 5: 275.

organic acids, but was readily thrown out of solution as a jelly by a trace of mineral acid or alkaline earth salts. The jelly was more soluble in water than the pectin (pectic acid) from currants.

Mulder^a and Regnault^b reported combustions of the metallic salts of pectins and pectic acid. Mulder concluded that pectic acid prepared by dissolving in alkali and precipitating with acid differed from pectin only in its higher ash content.

Frémy^c contributed an important article treating of the difficulties involved in this field of work and of the mutations of the pectin bodies. Pectin was prepared from fruit juices by first boiling to coagulate albuminous matters, filtering, and then repeatedly purifying by precipitating with alcohol and dissolving in water. The resulting body was white and soluble in water. It proved to be difficult to burn quantitatively on account of its ash, which retained carbon dioxide; so its lead salt was prepared and burned, the results indicating the formula $C_{24}H_{34}O_{22}$. Boiling this pectin with water increased the amount of lead which would combine with it, an increase of "saturation capacity."

The author states that pectins do not yield sugar on hydrolysis. Pectic acid dissolved in dilute potassium hydroxid would no longer precipitate on adding acid, the salt of metapectic acid having been formed. From the free meta acid, neutral lead acetate precipitated a salt much richer in lead than the lead salt of pectic acid. The free acid had an acid taste and was deliquescent. Long standing with caustic potash reduced an acid of still greater "saturation capacity" and more acid taste. Dilute acids effected a similar change in pectin and pectic acid (see Chodnew, p. 71). The author considered that the original pectin became hydrated in the above treatments and could in this way combine with more lead.

A discussion is given concerning the changes in the cell wall as fruits ripen, the wall becoming thinner and the fruit less acid. The presence of an insoluble mother substance of pectin-forming material, later named pectose, residing in the cell walls of unripe fruits, is suggested, as a result of experiments on a fruit marc, which with boiling water yielded only small quantities of pectin, but with dilute acid gave it in abundance.

Poumarede^d denied the existence of pectic acid in plants. He considered pectin to be an organized tissue, and pectic acid a reaction product.

Poumarede and Figuier^e considered pectin and "lignin" (cellulose) to be identical.

^a Ann. Chem. (Liebig), 1838, **28**: 280.

^b J. pharm. chim., 1838, **24**: 201; J. prak. Chem., 1838, **14**: 270.

^c Ibid., 1840 [2], **26**: 368.

^d Comptes rend., 1839, **9**: 660.

^e Ibid., 1846, **23**: 918.

Fromberg^a corroborated some of Frémy's work. Beet marc was boiled with dilute sodium carbonate in order to extract the pectin as the sodium salt of pectic acid, and the acid was precipitated by addition of excess of hydrochloric acid to the solution. On boiling a solution of pectic acid made faintly alkaline, a solution of the salt of metapectic acid was usually formed, from which no precipitate formed on acidifying.

Chodnew^b added materially to this literature of the pectin bodies. He considered that little was established in regard to their properties or constitution.

Pectin was prepared from pears, which were ground up, boiled, and the juice filtered first through linen, then through paper. The bright filtrate was precipitated with alcohol, and the filtrate washed with alcohol and ether and squeezed out, whereby it lost its gelatinous character and became like woody fiber (Holzfaser). This pectin was soluble, easily powdered, neutral in reaction, and gave no precipitate with calcium or barium chlorid, or with ammonium hydroxid. Lead acetate, basic lead acetate, copper sulphate, limewater, and potassium hydroxid gave the gelatinous precipitates. This pectin contained from 8.5 to 8.76 per cent ash.

Pectin from apple juice was studied in a similar way. It was also high in ash, but was largely freed from ash by precipitating by alcohol from an acid solution. This pectin gave the combustion figures: Carbon, 43.70 and 43.79; hydrogen, 5.63 and 5.41; oxygen, 50.67 and 50.80; and differed from pear pectin by not dissolving clear in water. Pectin is given as $C_{28}H_{21}O_{24}$.

Pectic acid was studied, the author first giving a résumé of previous work, particularly that of Frémy. It was prepared as follows, mostly from white beets: The marc was boiled with dilute potassium hydroxid for a quarter to half an hour, and filtered first through cloth and then through paper, the last filtration being very slow. It was considered better, after filtering through cloth, to precipitate the free acid and dissolve in ammonium hydroxid, which yields easily filterable solutions. The pectic acid was precipitated by hydrochloric or nitric acid washed with acidulated water, then with water containing alcohol, and finally with alcohol, and pressed out by hand. Alcohol is necessary in the wash water, for as the mineral acid diminishes in concentration, the pectic acid begins to stop up the pores of the filter. With alcohol the precipitate gradually lost its gelatinous property and became like woody fiber. It was colorless and easily powdered. By long drying at 120° C. it became yellow-colored. It was obtained nearly free from ash by repeatedly precipitating with alcohol from water solution made slightly acid with nitric acid. So prepared it burned without swelling, yielding 1 per cent of ash, which consisted

^a Ann. Chem. (Liebig), 1843, 48: 56.

^b Ibid., 1844, 51: 355.

chiefly of iron phosphate. It was insoluble or only slightly soluble in hot water, but readily dissolved in alkali to a clear solution.

The author discussed the difficulties in preparing pure metallic derivatives for analysis, which he considered to be best prepared from the alkali salt of pectic acid and a soluble salt of the metal. It is difficult to avoid inclosing excess of alkali or salt in the resulting jellies. This source of error was removed by squeezing out the salts and washing with water. The author so prepared and analyzed the calcium, barium, sodium, and silver salts, obtaining the formula $C_{28}H_{20}O_{26}$.

Pectinic acid was named and studied by Chodnew, who obtained it by boiling beet marc with hydrochloric acid, precipitating by alcohol, and washing with alcohol and ether. It differed from pectin in having a slight acid reaction. This author discussed the question of whether pectic acid exists as such in plants. Beet marc yielded no pectic acid to ammonium hydroxid, so that pectic acid may exist here in combined form, perhaps joined to calcium. But the marc after twice boiling out with hydrochloric acid (removal of pectinic acid?) still yielded to potassium hydroxid a body which on precipitating with acid showed all the properties of pectic acid. The author considered then that there existed in beet marc, not pectic acid, but a mixture of pectinic acid and a newly found acid—überpectic acid—which he stated was changed by potassium hydroxid into pectic acid, and was insoluble in ammonia. These two pectin bodies, pectinic acid and überpectic acid, he considered to be the sources of pectic acid as usually prepared. Similar results were obtained from apples, pears, and red and yellow beets. It was suggested that Frémy's acid, obtained from unripe gooseberries by boiling with acid, might be pectinic acid.

In support of the theory that pectin bodies exist combined with calcium in beet marc, the author described an experiment in which beet marc was allowed to stand with dilute hydrochloric acid. It became soft and translucent. On washing and adding a very little limewater, it regained its opacity and harshness.

Metapectic acid (cf. Frémy, p. 69), prepared by Chodnew by boiling pectin with potassium hydroxid, gave precipitates with acids and salts on standing, and a jelly with alcohol. Its lead salt treated with hydrogen sulphid gave a black colloidal solution from which it was impossible to separate the lead sulphid. Its properties differed from those given by Frémy in being not a penta-valent acid, not deliquescent in the air, and forming no soluble salts with calcium or barium. Pectic acid boiled for two hours with dilute mineral acid did not dissolve entirely to metapectic acid as Frémy states. The author carefully described the phenomena which occurred as the acid was boiled with dilute sulphuric acid salts. The solution soon became colored red, and reducing with silver and copper; formic acid and carbon dioxid were formed in small quantity, the former recognized by its odor, and the latter by limewater. As

the action progressed and the concentration increased, the odor of caramel appeared, but if the volume was kept constant the solution remained nearly colorless. There was nearly always left a black residue which consisted in part of pectic acid. This was soluble in alkali, from which acid threw it down in brownish flocks. From the reaction product, sulphuric acid was removed by barium carbonate. The filtrate was concentrated to a sirup and treated with alcohol, which yielded an abundant precipitate. The filtrate from the alcohol precipitate, evaporated to dryness, consisted largely of sugar, recognized by the crystalline form of the compound with sodium chlorid, reducing property, and by a weak fermentation. The presence of sugar explained the formation of the black substance, a material of the nature of humic acid. Boiling with sulphuric acid also gave rise to a peculiar odor, like that of benzoic acid.

This author notes that pectins disappear during the ripening of fruits; for example, pears containing much pectin when stored in the fall yielded only 0.5 gram from 100 pears in the spring. It is suggested that the pectins may form malic acid when they disappear as pectins.

Frémy^a now published an elaborate paper on the pectin bodies. Pectose was considered to be a substance analogous to starch. Pectin was prepared by boiling a fruit marc or a root marc with malic or citric acid, and also by precipitating a ripe fruit juice with alcohol. Its properties are given—neutral, soluble in water, insoluble in alcohol, not colored by iodine, not precipitated by neutral lead acetate, and inactive to polarized light.

Boiling pectin with water gave parapectin, which only differed from pectin in being precipitated by neutral lead acetate. Boiling pectin with dilute acid gave metapectin, like pectin and parapectin except that it was slightly acid and precipitable by barium chlorid. Prolonged boiling with acids gave parapectic acid; the action of pectase (an enzyme analogous to diastase) on pectin gave soluble pectosic acid which gelatinized. Alkalis converted pectin into pectic acid, soluble in alkalis and precipitating on adding acid. Heating pectic acid to 200 °C. gave pyropectic acid. The author noted great difficulty in obtaining pure pectin. Further changes and other pectin bodies were described in an unsatisfactory way. The methods employed were not described and practically no experimental data were given. The work of Chodnew received no notice. The formation of sugar by the acid hydrolysis of pectins is denied, since, though the solutions are reducing, they are not active to polarized light and are not fermentable.^b A list of pectins with proposed formulas is contained in the paper.

^aJ. pharm. chim., 1847, [3] 12: 13; Comptes rend., 1847, 24: 1046; Ann. Chem. (Liebig), 1847, 64: 383.

^bThe fact that pentoses undergo alcoholic fermentation with difficulty was discovered later. See W. E. Stone, Amer. Chem. J., 1891, 13: 73.

Another paper by Frémy^a appeared in 1848. The facts contained in the previous paper are retold, and new ones are given. The article ignores the work of every other chemist working in the field, and appears thirty-five years later, practically without change, in the *Encyclopédie Chimique*.

Neubauer^b reported concerning arabin, precipitated by alcohol from gum arabic solution (found by Scheibler to be identical with metapectic acid).

Frémy^c obtained metapectic acid by boiling beet marc with milk of lime.

Stude^d published work which was largely confirmatory of work already done by others. The existence of pectose was denied, the author believing that the pectin bodies in tissues existed as calcium pectate. No data of any kind are given in the paper.

Scheibler^e published two papers of much interest in which the formation of sugar from pectin is described. Metapectic acid was prepared from beet marc, and this by acid hydrolysis yielded a crystallizable sugar which he called arabinose. This was followed in 1873^f by a report of further work dealing with arabinose and metapectic acid. The sugar was the same whether prepared from beet marc or from gum arabic. The method of preparing metapectic acid used by him was as follows:

Beet pulp was repeatedly washed and macerated in cold 85 per cent alcohol. The pressed-out residue was thrown into boiling water, alcohol boiled out, potassium hydroxid added to a strong alkaline reaction, and the solution heated for a long time on a water bath. The product was then filtered, saturated with carbon dioxid, concentrated by evaporation, filtered, and the filtrate acidulated with acetic or hydrochloric acid. The pectic acid was then precipitated with alcohol and the crude product purified by repeated solution in water and precipitation with alcohol. Finally, the concentrated aqueous solution was poured into a small high cylinder, a little alcohol was added, and the mixture was allowed to stand several weeks. During this time there was formed a precipitate which carries nearly all the ash, and the filtrate yielded arabic acid of fine quality.

Girard^g determined the pectin in gum tragacanth.

Kirchner and Tollens^h hydrolyzed quince gum by boiling with 1.25 per cent of sulphuric acid for varying periods of time. In one exper-

^a Ann. chim. phys., 1848, [3] 24: 5; J. f. prak. Chem., 1848, 45: 385; Ann. Chem. (Liebig), 1848, 67: 257.

^b J. prak. Chem., 1854, 62: 193.

^c Compt. rend., 1859, 49: 561.

^d Ann. Chem. (Liebig), 1864, 131: 241.

^e Ber. d. chem. Ges., 1868, 1: 58, 108.

^f Ibid., 1873, 6: 612.

^g Ibid., 1875, 8: 340.

^h Ann. Chem. (Liebig), 1875, 175: 205.

iment the time of boiling was seven hours, and the reaction product was examined as follows: It was filtered and the insoluble residue on the filter weighed. Sulphuric acid was removed from the filtrate with barium carbonate, and sugar and soluble solids were determined. They found 28.3 per cent of nonhydrolyzed material, 39.8 per cent of sugar (calculated as dextrose), and 46.36 per cent of gum; in all, 114.46 per cent.

Reichardt^a prepared from carrots pararabin, a body somewhat different from Scheibler's arabic acid, but readily converted into it, and showing a carbohydrate percentage composition of $C_{12}H_{22}O_{11}$.

Barfoed^b studied changes of various arabic acids from soluble into insoluble form by standing in alcohol or on drying.

Scheibler^c found that beet marc, previously extracted with alcohol, yielded to water a body which could be obtained ash free, and which possessed a rotatory power of over $+200^\circ$.

Von Lippman^d described a body, γ -galactan, of high rotatory power from the scums of sugar manufactured from unripe beets, possibly very similar to the metapectic acid obtained by Scheibler.

Muntz^e suggested that the galactose complexes so widely distributed in plants were probably the sources of the galactose radical in lactose in milk.

Battut,^f Chevron,^g and Pellet^h discussed the influence on the polarization of sugar of the dextrorotatory pectin dissolved on boiling sugar-free beet marc with water. Basic lead acetate precipitates it completely, thus disposing of the question of its interference with polarimetric measurements.

Weisbergⁱ confirms the view of Scheibler that gelatinous hot water extract from beet marc exhausted by alcohol was a pectin. On heating with water the acidity of the solution gradually increased. By sulphuric acid hydrolysis of the pectin, he also obtained a furfural yielding sugar (arabinose).

Wohl and Van Niessen^k discuss the work of Scheibler. Pectins of beet marc were considered to be insoluble in water, unless rendered soluble by hydrolysis, and to be slowly changed to soluble forms by hot water, and much more rapidly by acids and alkalis. For example,

^a Ber. d. chem. Ges., 1875, **8**: 807.

^b J. prak. Chem., 1875, [2] **11**: 186.

^c Neue Zts. f. Rübenz., 1879, **3**: 341.

^d Ber. d. chem. Ges., 1887, **20**: 1001.

^e Ann. chim. phys., 1887 [6], **10**: 566.

^f Sucre indigène, 1888, **32**: 285, 311, 333, 357, 415, 456.

^g Neue Ztg. f. Rübenz., 1888, **20**: 169. Sucre belge, 1888, **13**: through Chem. Ztg. R., 1888, **12**: 82.

^h Sucre indigène, 1888, **32**: 390.

ⁱ Neue Zts. f. Rübenz., 1888, **21**: 325.

^k Zts. Ver. d. Zucker-Ind., 1889, **39**: 924.

water dissolved about one-third of beet marc in about thirty hours, while dilute oxalic acid dissolved nearly 45 per cent in four hours, heating on the water bath in each case. The method used for obtaining mucic acid from marc is as follows: 35 grams of beet pulp containing 4.3 per cent of marc were extracted with alcohol, the alcohol removed, and the marc thoroughly dried. The marc was then heated with 17 cc of nitric acid (sp. gr. 1.15) for two hours in a boiling water bath in a 100-cc flask. The reaction product was cooled, diluted to mark, filtered, and 50 cc evaporated to 3 cc. After standing forty-eight hours, 94.5 mg of mucic acid had separated, equal to 0.53 per cent of original pulp, or 12.4 per cent, calculated to marc, which equals 16.2 per cent of galactose or 14.6 per cent of galaetan.

Herzfeld^a considered pectins as combinations of araban and galactan, not separable by known means, and recognized by yielding furfural on the one hand and mucic acid on the other. He was able to concentrate the furfural yielding a complex by precipitating the ammonia solution of parapectin with calcium chlorid, the calcium salt yielding as high as 40 per cent of furfural. From ripe oranges he obtained an inactive pectin which was, however, precipitable by neutral lead acetate, distinguishing it from Frémy's pectin.

Bertrand and Mallévre^b discussed the effect of the ferment pectase on pectin, and the wide distribution of that enzym in plants. It is especially abundant in leaves, and it has been possible to prepare it from this source. It can only coagulate pectins in the presence of alkaline earth salts, forming salts of pectic acid. In acid fruits it is present in soluble form, but its action is inhibited by the free acid.

Ullik^c found pectic acid to have a high rotatory power (about $[\alpha]_D = +186^\circ$ to 300°) and to form easily soluble, alkali salts dialysing readily, while the other salts are insoluble and gelatinous. He separated up to 80 per cent of mucic acid, but noted that pectic acid from different sources and prepared by different methods behaved very differently in regard to yield of mucic acid; some giving the above high percentage, while others gave very little, and still others no mucic acid whatever. Those yielding high percentages of mucic acid showed the highest rotatory power (up to $[\alpha]_D = +300^\circ$), and on hydrolysis passed over principally into galactose, while those showing low percentages of mucic acid gave exclusively or almost exclusively arabinose. Such characteristics are indicated by the author in case of pectin from beet marc.

Sugar-free beet marc was allowed to stand several days with 1 per cent of hydrochloric acid, then pressed out, and the filtrate concen-

^aZts. Ver. d. Zucker-Ind., 1891, **41**: 295, 667.

^bCompt. rend., 1894, **119**: 1012; 1895, **120**: 110; 1895, **121**: 726.

^cÖsterr.-Ung. Zts. Zucker-Ind. Landw. **21**: 546; **23**: 268, through Chemie der Zuckerarten, von Lippman, 1895, p. 927, 928.

trated at the lowest possible temperature. Alcohol was added and resulting gelatinous precipitate after filtering off was dissolved in water. After digesting for one hour with 1 per cent hydrochloric acid at 60° C., and filtering, two precipitates were formed on fractional precipitation of the filtrate with alcohol. The first, after repeated solution and precipitation with water and alcohol respectively, was a white weakly acid mass, precipitated by barium chlorid and lead acetate, of strongly dextro-rotatory power ($[\alpha]_D = +167.4^\circ$), and yielding 20 per cent of mucic acid on oxidation. The second, after purification in the same way, was an amorphous white substance, precipitated by lead acetate, but not by barium chlorid, showing less dextro-rotation ($[\alpha]_D = +123.8^\circ$), and gave no mucic acid on oxidation, but yielded with phloroglucin and hydrochloric acid an intense color reaction denoting pentoses or pentosans.

Tromp de Haas and Tollens^a gave the ultimate composition and products of hydrolysis by sulphuric acid of pectins from many sources. Their results showed that the relation of hydrogen and oxygen in these bodies was nearly 1 to 8, as required by the carbohydrate formula, and that the pectin bodies which they studied contained no complex which gave rise to dextrose, but that complexes were present which yielded pentose sugars and galactose on hydrolyzing with acids.

Tollens^b in a later paper says that pectin bodies may probably be regarded as glucosides, since the acid reaction, combination with bases, and slightly higher oxygen-hydrogen ratio of extracted pectins indicate the presence of carboxyl groups. The pectin in the plant may not have acid properties, but may exist as a lactone.

Andrlik^c discusses the action of dilute hydrochloric acid in the cold on beet marc. A pectin of specific rotatory power ($[\alpha]_D = +214.4^\circ$ to 220°) was extracted, and purified by repeated precipitation with alcohol. The longer the acid acted on the beet marc, the more insoluble in water was the pectin dissolved.

Bourquellot and Hérissé,^d and later Bourquellot^e alone, studied the extraction of pectins with hot water, and the effect of two enzymes on the dissolved bodies. A 1 per cent solution of pectins from gentian root was gelatinized by a solution of pectase within 40 minutes, and also by limewater, sodium hydroxid followed by hydrochloric acid, neutral and basic lead acetates, ferric chlorid, magnesium sulphate, and ammonium sulphates, but not by sodium sulphate. The pectin was not reducing and was dextro-rotatory ($[\alpha]_D = +82.3^\circ$). Acidified water extracted a more dextro-rotatory body ($[\alpha]_D = +145.3^\circ$).

^a Ann. Chem. (Liebig), 1895, **286**: 278.

^b Ibid., 1895, **286**: 292.

^c Zts. Zucker-Ind. Böhm., 1894, **19**: 101, through Chem. Centrbl. 1895, **66**: 1, 833.

^d J. pharm. chim., 1898, [6] **7**: 473; 1898, [6] **8**: 145; 1899, [6] **9**: 281.

^e Compt. rend., 1899, **128**: 1241.

They found that the soluble ferments produced by *Aspergillus niger* partially hydrolyzed the pectose of gentian root, converting it into pectin. Pectin was hydrolyzed (rendered noncoagulable) by diastase from malt, but not by saliva or by emulsin. The pectin was said to yield mucic acid and arabinose like that from beet marc. Bourquellot defined pectins as substances which dissolve in water, yield mucic acid, and are coagulated by limewater, baryta water, and by pectase. The solutions were optically active, contrary to the results of Frémy ($[\alpha]_D = 82.3^\circ$ to 194°). The ferment in malt which dissolves pectin he called pectinase. Pectase and pectinase, added together to a pectin solution, caused coagulation, then solution, similar to the effects of rennet and trypsin on casein.

Javillier,^a using the methods of Bourquellot and Hérissé, obtained corroborating results with quince pectin. It was strongly dextro-rotatory ($[\alpha]_D = 188.2^\circ$), gave arabinose and mucic acid, and behaved toward malt diastase like the pectins obtained by Bourquellot.

Votocěk and Šebor^b obtained from beets, by the treatment with alkali, an arabic acid which they determined to be not a homogeneous compound, since different preparations showed varying rotatory powers. Other evidence that the arabic acid was a mixture of similar substances was that varying quantities of arabinose and galactose were formed on hydrolysis of different preparations, and by hydrolyzing the acetylation product the original acid was not regained, but the products possessed different rotatory powers and contained different proportions of the groups which give rise to arabinose and galactose. A very pure glucosazone was obtained from the hydrolysis product of the arabic acid, so that three complexes may be present—araban, galactan, and glucose.

Bauer^c obtained various sugars from pectins from different sources—galactose from pear pectin, xylose from apple pectin, and other sugars not positively identified from orange peel.

Widtsoe and Tollens^d reported arabinose, xylose, and fucose in gum tragacanth, which Girard^e had found to be 60 per cent pectin.

Cross^f considers that pectin may be ligno-cellulose free from incrusting materials, and suggests that it would be well to try the methods used for separating cellulose bodies on the pectins.

^a J. pharm. chim., 1899, [6] 9: 163 and 513.

^b Zts. Zucker-Ind. Böhm., 1899, 24: through Chem. Centrbl., 1899, [2] 70: 1022, through J. Chem. Soc., 1900, 78: 1, 208.

^c J. prak. Chem., 1891, [2] 43: 112; Landw. Versuchs-Stat., 1892, 41: 477; 1894, 43: 191; Verh. Vers. Deutsch. Ntf. u. Ärzte, 1900, II, 1, Hälfte, 99, Aachen, through Chem. Centrbl., 1901, [2], 72: 196.

^d Ber. d. chem. Ges., 1900, 33: 132.

^e Ibid., 1875, 8: 340.

^f Ibid., 1895, 28: 2609.

Mangin^a contributed a comprehensive review, referred to at the beginning of this paper, and studies which appear to be worth corroborating at least by the microchemist, and if proved to be reliable, they may be used in connection with chemical investigation. Mangin considers that pectic compounds are constant constituents of cell membranes; that pectose itself may be a mixture of several similar compounds or a single body; that pectose seems to be elaborated earlier than cellulose in young tissue, and forms the intermedullary layer in mature tissue; that it is not elaborated from cellulose; and that soft parenchymatous tissue is essentially characterized by a very close association of cellulose and of pectins.

Many coloring agents are stated to dye the pectins in plant tissues and differentiate them from cellulose but not from nitrogenous matters nor from lignin, suberin, or cutin, so that the number of suitable dyes is small. These dyes are the safranines, methylene blue, "bleu de nuit," and naphthalene blue R in crystals. Safranine is said to color the nitrogenous bodies and lignin a cerise red, while the pectins are colored a yellow orange. Methylene and "bleu de nuit" color nitrogenous bodies and lignin a beautiful blue, while the pectins are colored a violet blue best seen by lamplight. A mixture of naphthalene blue R in crystals and acid green J. E. E. E. (poirier) (equal parts of a 1 per cent solution of each) colors pectins violet and other bodies green. Ruthenium red is also recommended.

The above review does not nearly do justice to Mangin's contributions. Since the methods used are distinctly microchemical, however, a more extended notice does not seem necessary in this connection. Much of the material found in Mangin's review is also found in an article by Reynolds Green,^b who discusses the pectin bodies from the botanist's point of view.

Most of the facts brought out in the above review are tabulated, for convenience of reference, in Table VII (see p. 80).

^aCompt. rend. 1888, **107**: 144; 1889, **109**: 579; 1890, **110**: 295; 1893, **116**: 653; J. bot. 1891, **5**: 400-440; 1892, **6**: 12, 206, 235, 363; 1893, **7**: 37, 121, 325.

^bScience Progress, 1896, **6**: 344.

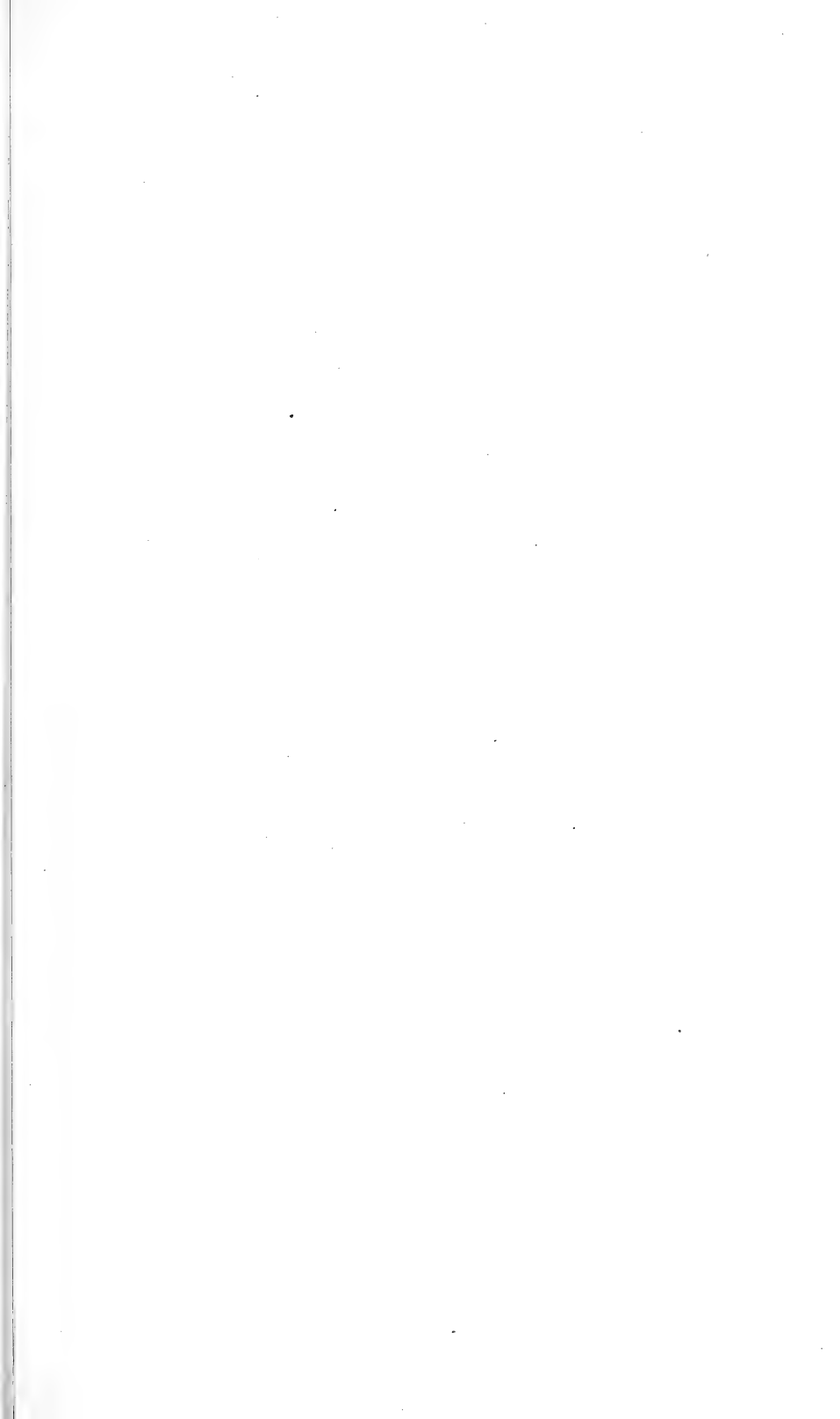


TABLE VII.—*Tabular résumé of*

Name.	Author.	Date.	Page in this review.	Source.	Preparation.
Pectin.....	Braconnot.....	1825	67	A very wide range of plant tissues.	The marc of the tissues is extracted with hot dilute alkali after exhaustion with dilute hydrochloric acid. The product is precipitated by acidifying the solution.
Do.....	Vauquelin	1829	68	(a) From carrot juice and (b) from the marc of carrot.	(a) By precipitating with alcohol; (b) by extracting with dilute alkali and acidifying.
Do.....	Mulder	1838	69		
Do.....	Frémy.....	1840	69	Fruit juices	The juice is clarified by boiling, then precipitated by alcohol. The product is then purified by repeated solution in water and precipitation with alcohol.
Do.....	Chodnew.....	1844	70	Pears, apples	The crushed fruit is boiled with water, the product filtered, and the filtrate precipitated with alcohol.
Do.....	Poumarede and Figuiér.	1846	69		
Do.....	Frémy	1847	72	Fruit mares and fruit juices.	From mares by boiling with solutions of organic acids; from juices by precipitating with alcohol.
Do.....	Stude.....	1861	73		
Do.....	Girard.....	1875	73	Gum tragacanth.....	
Do.....	Scheibler.....	1879	73	Beet marc	By boiling with water
Do.....	Weisberg	1888	74do.....do.....
Do.....	Wohl and Van Niessen.	1888	74do.....	Made soluble by boiling with water.
Do.....	Herzfeld.....	1891	75	Beet marc and oranges.	From beet marc by heating with water; from oranges by precipitating the clear juice with alcohol.

the literature of the pectin bodies.

Properties.					Remarks.
Solvents.	Precipitants.	Polarization.	Results of acid hydrolysis.	Other properties.	
Alkaline solutions.	Metallic salts; alcohol; sugar.			Mucic and oxalic acid formed on oxidation with nitric acid.	The product obtained by Braconnot is probably the pectic acid of later workers.
(a) Water; (b) alkali solutions.	(a) Alcohol; (b) acids.				The product from the juice (a) was probably the pectin and that from the marc (b) the pectic acid of later workers.
				Combustion figures given for pectin and pectic acid.	Pectic acid was believed to differ from pectin only in its higher ash content.
Water			Does not yield sugar; metapectic acid formed.	Alkali hydrolysis yielded the salt of metapectic acid. Combustion figures of lead salt given. Boiling with water increased the amount of lead which would combine with the pectin.	The existence of a mother substance in the cell walls of fruits, which slowly passes into pectin as the fruits ripen, is suggested.
do	Lead acetate; basic lead acetate; copper sulphate; lime water; potassium hydroxid.			Neutralin reaction, easily powdered. Combustion figures given. Apple pectin differs from pear pectin in not dissolving in water to form a clear solution. Pectins disappear as fruits ripen.	
Water	Alcohol	Inactive	Does not yield sugar, but gives metapectin and parapectic acid.	Neutralin reaction, not precipitated by lead acetate. The enzyme pectase produces pectonic acid. Solutions of alkalis change pectin to pectic acid. Pyropectic acid is produced on heating to 200° C.	Pectin was considered to be identical with cellulose. No other authors agree with these.
Water		$[\alpha]_D^{20} = +$			A discussion only; no data given.
do	Alcohol; basic lead acetate.	200°.	Arabinose	On heating the water solution its acidity gradually increased.	
			do	Mucic acid formed from the beet marc indicating presence of galactan.	
Water	Alcohol; lead acetate.	From oranges inactive.	do	Mucic acid	Pectin considered to be a mixture of arabans and galactans.

TABLE VII.—*Tabular résumé of the*

Name.	Author.	Date.	Page in this re- view.	Source.	Preparation.
Pectin.....	Bertrand and Mallèvre.	1894	75		
Do.....	Tromp de Haas and Tollens.	1895	76		
Do.....	Tollens.....	1895	76		
Do.....	Andrlik.....	1895	76	Beet mare.....	Cold dilute* hydrochloric acid.
Do.....	Bourquellot and Herissey.	1898	76	Gentian root.....	Extraction with hot water.
Do.....					
Do.....	Javillier.....	1899	77	Quince.....	do.....
Do.....	Bauer.....	{ 1891 1892 1894 1900 }	77	Pears, apples, orange peel.	
Do.....	Cross.....	1895	77		
Do.....	Mangin.....	{ 1888 to 1893 }	78		
Pectic acid....	Frémy.....	1840	60		
Do.....	Fromberg.....	1843	70	Beet mare.....	Boiling with sodium car- bonate.
Do.....	Chodnew.....	1844	70	do.....	Boiling with dilute potassi- um hydroxid, then fil- tering and precipitating with hydrochloric acid.
Do.....	Frémy.....	1847	73	Pectin.....	Treatment with alkali.....
Do.....	Ullik.....		75		
Pectinic acid..	Chodnew.....	1844	71	Beet mare.....	Boiling with dilute hydro- chloric acid and precipi- tating with alcohol.

Literature of the pectin bodies—Continued.

Properties.					Remarks.
Solvents.	Precipitants.	Polarization.	Results of acid hydrolysis.	Other properties.	
				Coagulated by pectase in presence of salts of alkaline earth metals.	
			Pentose sugars and galactose.	Hydrogen to oxygen ratio is nearly 1 to 8. Combustion figures given. Author considers that pectin may be regarded as a glucoside.	
Water	Alcôhol	$[\alpha]D = +214.4^\circ$ to 220° .			
do	Coagulated by pectase, limewater, sodium hydroxid followed by hydrochloric acid, neutral and basic lead acetate, ferric chlorid, magnesium and ammonium sulphate.	$[\alpha]D = +82.3^\circ$ and $+188.2^\circ$.	Arabinose	Mucic acid formed. Pectin hydrolyzed by malt diastase, not by saliva or emulsin.	Pectins defined as bodies which dissolve in water, yield mucic acid, coagulated by limewater, baryta water, and by pectase, and which are dextro rotatory.
		$[\alpha]D = 188.2^\circ$.	do	Mucic acid formed. Hydrolyzed by malt diastase.	
			Galactose and xylose.		
					Discussion only: pectins may be lignocellulose free from incrusting materials.
					Discussion and microscopical work. Pectin a constant fundamental constituent in young tissues.
Alkaline solutions.	Acid solutions			Prolonged treatment with alkali gave metapectic acid. No precipitate would form on acidifying.	
Alkalis	Acids			Prolonged boiling in dilute alkali gave a solution from which nothing would precipitate on acidifying, metapectic acid having been formed.	
Alkaline solutions.	do				
		$[\alpha]D = +186^\circ$ to 300° .		Forms easily soluble alkali salts. Yields up to 80 per cent of mucic acid.	
Water	Alcohol			Possesses a slight acid reaction.	

TABLE VII.—*Tabular résumé of the*

Name.	Author.	Date.	Page in this re- view.	Source.	Preparation.
Überpectic acid.	Chodnew.....	1844	71	Beet marc	Beet marc, after exhausting with dilute hydrochloric acid, was treated with al- kali.
Pectose.....	Frémy.....	1847	72	Plant tissues.....
Parapectin	do	1847	72	Pectin	Boiling with water
Metapectin	do	1847	72	do	Boiling with dilute acid
Parapectic acid	do	1847	72	do	Prolonged boiling with acids.
Pectosic acid	do	1847	72	do	Treatment with the en- zym pectase.
Metapectic acid	Chodnew.....	1844	71	do	Boiling with alkali
Do.....	Frémy.....	1859	73	Beet marc	Boiling with milk of lime..
Metapectic acid (arabic acid).	Scheibler.....	1868	73	do	Heated with potassium hy- droxid, filtered, acidu- lated, and precipitated with alcohol.
Pararabin	Reichardt	1875	74	Carrots

literature of the pectin bodies—Continued.

Properties.					Remarks.
Solvents.	Precipitants.	Polariza- tion.	Results of acid hydrolysis.	Other properties.	
	Neutral.				Regarded as the substance which gives rise to the pectin bodies.
	Lead acetate:				
	barium				
	chlorid.			Like pectin and parapectin, save slightly acid.	
	Alcohol; acids:		A sugar formed.		
	salts.				
			A r a b i n o s e formed.		
					Very similar to Schei- bler's arabic acid.

From the foregoing review pectin bodies would be defined as substances of undetermined function, very widely distributed in plant tissues. Pectin bodies occur both in the juice and marc, i. e., in soluble and insoluble forms. The latter form, according to the work of Scheibler, Wohl, Van Niessen, and others, and from the work done in the Bureau of Chemistry, seems to be resolved into soluble forms by boiling with water. The solutions possess considerable viscosity, the property of forming jellies with precipitants—such as alcohol, sugars, solutions of salts, and pectase—and usually rotate polarized light to the right. The rotating power of the different pectin bodies appears to vary considerably. The distinctions laid down by Frémy as existing between the pectin bodies seem to be based on uncertain physical properties^a and on the amount of lead with which they will combine. Criteria adopted by later workers are based mainly on chemical behavior and action on polarized light.

Chemically, pectin bodies are characterized by yielding reducing sugars, furfural, and mucic acid in widely varying amounts, according to the source of the pectins and the method employed in isolating them. These variations may be largely due to varying degrees of hydration and to impurities in the pectin bodies examined. Differences in the pectin bodies themselves, however, are indicated by the results of Ulrik and Herzfeld. Pectins are profoundly changed by alkalis with the formation of salts of the so-called pectic acid, the free acid being insoluble in water. Acid groups appear to be formed even by treatment with very dilute alkali for a very short time, so that extraction with alkali of pectins from plants does not recommend itself to the writers when a study of the dissolved material is desired.

The most important problem appears to be the quantitative determination of the pectin bodies occurring in a given tissue, because such a method could be used to determine the function of the material in plants—whether, for example, it is a reserve material, a by-product, is used for structural purposes, or has all three functions or two of them; whether the nature of the pectin body changes with the growth or age of the tissue, or possesses a practically constant composition; whether the pectin bodies obtained from different sources are identical, are mixtures of the same substances (such as araban and galactan) in varying proportions, or are inherently different.

It is of interest to note that all pectin bodies thus far studied have been derived from the softer tissues. The harder woody material has not been considered.

^aJ. prak. Chem., 1884, **30**: 370. Neue Ztg. Zucker-Ind., 1885, **14**: 151.

In this connection the following quotation from Cross and Bevan^a is of interest:

It appears, therefore, generally, that the pectic group are compounds of carbohydrates of varied constitution with acid groups of undetermined constitution, associated together to form molecular complexes, more or less homogeneous, but entirely resolved by the continued action of simple hydrolytic agencies; and the pectocelluloses are substances of similar character in which the carbohydrates are in part replaced by nonhydrolyzable celluloses. The general characteristics of the pectocelluloses are therefore these: they are resolved by boiling with dilute alkaline solutions into cellulose (insoluble) and soluble derivatives of the noncellulose (pectin, pectic acid, metapectic acid); they are gelatinized under the alkaline treatment; they are "saturated compounds," not reacting with the halogens, nor containing any groups immediately allied to the aromatic series.

Later in the same volume, page 221, Cross and Bevan refer to the parenchymatous tissue of fruits, fleshy roots, etc., as being typical pectocelluloses.

This suggests the possibility that all insoluble pectin bodies occurring in the vegetable world are really in combination with cellulose and belong to the group of pectocelluloses. This idea is in part borne out by the results previously published by several writers and confirmed by the results given on page 88, in which it is seen that insoluble pectin bodies are changed to soluble form by boiling with water. If such insoluble pectin bodies, usually called pectoses, are really pectocelluloses, this action by which they are converted into soluble form is really a splitting off of the pectin group from the cellulose complex.

In this connection should be noted the recent work by Mangin,^b in which attention is called to deposits which that writer considered to be calcium pectate between the cell walls of plant tissues. His conclusions, however, have recently been disputed by Devaux,^c who asserts that this insoluble deposit was not really calcium pectate but true pectose. If it is found that the substance now known as pectose is really a pectocellulose, it is suggested by the writers that the latter term be employed to designate it and that the use of the term pectose, which is a misnomer and altogether misleading, be discontinued.

ANALYSES OF APPLE MARC.

PREPARATION OF SAMPLE.

Fully ripe Rhode Island Greening apples were taken from cold storage, wiped off, quartered, cores and bruised places removed, and passed through a meat grinder. The pulp was then exhausted with water by pressing out by hand in cloth bags with successive portions

^a Cellulose, 2d ed., 1903, p. 217.

^b Loc. cit. (see p. 78).

^c Mémoires de la société des sciences physiques et naturelles de Bordeaux, 1903 [6], 3: 90.

of water until the wash water gave no test for reducing sugar. The resulting marc, amounting to 2.745 per cent of the ground fresh pulp, was spread out in shallow pans and placed on a steam radiator, and, after drying, was ground to a fine powder, exhausted with alcohol, and then with ether in a Soxhlet's extractor. The alcohol removed any sugar remaining in the marc, and the extractions which occur on the skins of apples, namely, apple wax and a white solid, apple vitin.^a Ether then removed very little from the marc, chiefly green coloring matter. The ether was evaporated spontaneously and the marc bottled.

RESULTS OF ANALYSIS.

Prepared as above, the marc was analyzed, with the following results:

TABLE VIII.—*Analysis of apple marc.*

Determinations.	Air-dry basis.	Water-free basis.
	<i>Per cent.</i>	<i>Per cent.</i>
Water <i>a</i>	14.04	
Cellulose <i>b</i>	33.82	39.33
"Starch" <i>c</i>	27.68	32.20
Pentosans <i>d</i>	22.79	26.51
Protein <i>e</i>	3.50	4.07
Ash96	1.12
Soluble in boiling water: <i>f</i>		
First hour	20.83	24.23
Second hour	8.52	9.91
Third hour	4.37	5.08
Fourth hour	2.67	3.10
Fifth hour	2.53	2.94
Sixth hour	1.92	2.23
Total	40.84	47.49
Soluble in 1 per cent sodium hydroxid by difference <i>g</i>	62.94	56.80
Residue from boiling-water treatment <i>h</i>	46.60	54.22
Residue from 1 per cent sodium-hydroxid treatment <i>i</i>	37.06	43.20

^a Dried at 100° to constant weight.

^b Method of Cross and Bevan, "Cellulose," p. 95.

^c No starch was present, but the marc hydrolyzed by hydrochloric acid, as in the method for starch (see p. 66), gave the above percentage of reducing sugar calculated as starch.

^d Bul. 65, Bureau of Chemistry, U. S. Dept. of Agr., p. 173; Cir. 7, Bureau of Chemistry, U. S. Dept. Agr., revised edition, p. 1.

^e N x 6.25.

^f Twenty grams of marc were boiled with 400 cc of water under a return condenser for successive periods of 1 hour each; the product was filtered after each boiling through a cloth bag, the residue washed and returned to flask, and the filtrate evaporated and weighed.

^g The actual amount dissolved may be somewhat higher than is indicated here, owing to hydration (see footnote "h").

^h It will be noted that the sum of the residue and material extracted by boiling water is greater than 100 per cent (101.71 per cent dry basis), probably indicating hydration of one or both of the products.

ⁱ Five grams of the air-dry marc were boiled with 200 cc of 1 per cent sodium hydroxid for one-half hour, this being the first treatment described for the determination of cellulose by the chlorination method described by Cross and Bevan.

It will be noted that 11.02 per cent (dry basis) is soluble in sodium hydroxid, and not dissolved by the hot-water treatment. But it is probable that longer treatment with boiling water would have removed larger amounts. It seems impossible to make sharp separations. It will also be noted that the lignin (removed by chlorin and sodium sul-

phite treatments in method for cellulose) amounts to but 3.87 per cent of the dry marc.^a

The pectin removed during the first hour of boiling was soluble in water. It yielded 46.58 per cent of pentosan and 54.6 per cent "starch" (^b) and showed a rotatory power of $[\alpha]_D = 71.2^\circ$ to 78.7° ; i. e., a 1.851 per cent solution polarized at 3.8° to 4.2° in a Schmidt and Haensch polariscope in a 10-cm tube. The pectin removed during the second hour gave 41.46 per cent of pentosan and 30.02 per cent "starch." It was impossible to get a solution clear enough to polarize. No mucic acid determinations were made.

Whether pectin in apple marc is a series of bodies of similar constitution but of different degrees of hydration, or whether it is a mixture of bodies of different kinds, was not determined. This could be found out by examining, by the criteria of yields of reducing sugar on hydrolysis with acid, pentosans, and mucic acid, the portions obtained by fractional extraction with boiling water from the tissue and by a similar examination of the products resulting from fractional precipitation of the water extract with alcohol. It is hoped that such studies may be made.

III. MICROSCOPIC AND MACROSCOPIC EXAMINATIONS OF APPLE STARCH.

MICROSCOPIC STUDIES.

SIZE OF STARCH GRAINS.

The presence of starch in apples is in itself no new consideration. Buignet^c in his work upon the apple failed to find starch present, although the filtered juice from green fruit was colored blue with iodine, which phenomenon was accredited to the presence of tannin. According to Lindet,^d the earliest mention of starch in apples was made by Grignon^e in 1887. Lindet himself describes the general features concerning the disappearance of the starch, and gives as the size of the grains 6 to 20 microns.

An examination of the starch as it normally occurs in the tissues will show that the grains are generally compounded, being composed of from 2 to 5 simple grains. In preparing samples of apple starch, the grating of the flesh usually results in tearing these compound grains apart, so that grains having a truncated form very greatly predominate, as is shown in Plate I.

^aThat is, 43.20 per cent was insoluble in 1 per cent sodium hydroxid and 39.33 per cent of cellulose was obtained, so that 3.87 per cent was removed by the treatments with chlorin and sodium sulphite.

^bSee footnote—c, page 88.

^cJ. phar. chim., 1859, (3) 36: 81-111.

^dAnn. agron., 1894, 20: 5-20.

^eLe Cidre Doin.

The individual grains are small and vary in size from 1 to 16 microns, though generally they are between 4 and 10 microns. Browne^a found the average size of the grains to be about 9 microns, but the measurements made in the Bureau of Chemistry show that the average varies widely, according to the condition of maturity of the fruit. In Table IX are given the results of measurements of grains from several varieties. An examination of these results will show that, generally speaking, the starch grains from within the core line are smaller than those from the torus flesh. In only three specimens, out of the 47 which were examined with this point in mind, did the reverse condition occur.

It is true, however, that in nearly ripe apples the figures might give a slightly erroneous idea, inasmuch as there does not appear to be so much decrease in the average size of the grains as might be expected. In this connection, however, it must be remembered that the number of starch grains has decreased very much, and the great majority of those which figured in earlier measurements have disappeared, leaving only a few stray grains behind. The smallest-sized grains which the writer has been able to identify and measure are those slightly less than 1 micron in diameter. The writer has also occasionally found what seemed to be stray starch grains remaining in apparently normal cells of apples which appeared fully ripe. Such grains are rare, however, and the cause for their persistence is not at all clear, though it is possible that it is due to a condition within the cells themselves, resulting in a vitality too low to carry on the ordinary metabolic change.

All the specimens of each variety were taken from the same orchard so that the variations arising from differences in climate and soil have been eliminated.

TABLE IX.—*Starch-grain dimensions.*

PRELIMINARY OBSERVATIONS (GROWING FRUIT).

Serial No.	Variety.	Date of examination.	Size of grains in torus flesh. ^b	Size of grains within core line. ^b
		1902.	<i>Microns.</i>	<i>Microns.</i>
.....	York Imperial.....	July 22	7.3 (2.4 to 11.2)	6.0 (2.4 to 11.2)
.....	Grimes.....	do	10.2 (6.0 to 16.0)	6.0 (2.4 to 11.2)

COMMON-STORAGE APPLES.

1129	Rhode Island Greening:			
	A.....	Sept. 17	5.5 (3.2 to 8.8)	2.9 (1.6 to 6.4)
	B.....	do	6.0 (2.4 to 9.6)	4.2 (1.6 to 8.0)
	C.....	do	4.9 (1.6 to 9.6)	4.2 (1.6 to 8.0)
	D.....	do	4.2 (1.6 to 9.6)	3.1 (0.8 to 6.4)
	Average.....		5.1 (1.6 to 9.6)	3.6 (0.8 to 8.0)

^a Pa. Dept. of Agr., bulletin 58.

^b The mean diameter of the starch grains in each specimen is given, followed by the minimum and maximum diameters in parenthesis; then the average of the mean diameters for all specimens examined on the same date is given, followed by the lowest and highest diameters found in the group.

TABLE IX.—*Starch-grain dimensions*—Continued.

COMMON-STORAGE APPLES—Continued.

Serial No.	Variety.	Date of examination.	Size of grains in torus flesh.	Size of grains within core line.
1127	Rhode Island Greening:		<i>Microns.</i>	<i>Microns.</i>
	A	Oct. 7	4.7 (2.4 to 8.0)	3.8 (1.6 to 7.2)
	B	do ..	4.6 (1.6 to 8.0)	3.7 (1.6 to 8.0)
	C	do ..	5.7 (1.6 to 9.6)	4.4 (0.8 to 8.0)
	Average		5.0 (1.6 to 9.6)	3.9 (0.8 to 8.0)
1121	Northern Spy:			
	A	Sept. 17	7.0 (2.4 to 14.4)	4.0 (1.6 to 7.2)
	B	do ..	6.7 (2.4 to 13.6)	4.2 (1.6 to 7.2)
	C	do ..	9.5 (1.6 to 12.0)	3.7 (1.6 to 5.6)
	D	do ..	8.1 (4.0 to 13.6)	4.8 (1.6 to 8.8)
	Average		7.8 (1.6 to 14.4)	4.2 (1.6 to 8.8)
1128	Northern Spy:			
	A	Oct. 7	5.3 (2.4 to 9.6)	3.7 (1.6 to 9.6)
	B	do ..	5.5 (1.6 to 11.2)	4.2 (1.6 to 11.2)
	C	do ..	6.4 (1.6 to 9.6)	4.7 (2.4 to 11.2)
	Average		5.7 (1.6 to 11.2)	4.2 (1.5 to 11.2)
1154	Northern Spy:			
	A	Nov. 8	5.1 (2.6 to 10.5)	5.4 (3.5 to 7.9)
	B	do ..	5.2 (1.7 to 8.8)	5.3 (2.6 to 9.6)
	Average		5.1 (1.7 to 10.5)	5.3 (2.6 to 9.6)
1122	Ben Davis:			
	A	Sept. 19	9.2 (3.2 to 15.2)	5.3 (1.6 to 12.8)
	B	do ..	9.0 (3.2 to 16.8)	3.9 (1.6 to 9.6)
	C	do ..	8.6 (4.0 to 13.6)	5.9 (2.4 to 10.4)
	Average		8.9 (3.2 to 16.8)	4.8 (1.6 to 12.8)
1145	Ben Davis:			
	A	Oct. 23	3.6 (0.8 to 8.0)	No starch present.
	B	do ..	4.6 (1.6 to 8.0)	No starch present.
	Average		4.1 (0.8 to 8.0)	
1146	Ben Davis ^a	Oct. 23	No starch present.	No starch present.
1153	Ben Davis	Nov. 7	3.5 (0.8 to 5.3)	3.1 (0.8 to 6.0)
1164	Ben Davis	Nov. 18	No starch present.	No starch present.
1123	Winesap:			
	A	Sept. 19	9.5 (4.8 to 12.8)	5.6 (2.4 to 14.4)
	B	do ..	9.6 (4.0 to 14.4)	8.8 (5.6 to 13.6)
	C	do ..	10.0 (2.4 to 16.0)	5.4 (2.4 to 11.2)
	Average		9.7 (2.4 to 16.0)	6.6 (2.4 to 14.4)
1126	Winesap:			
	A	Oct. 7	7.3 (1.0 to 11.0)	4.8 (1.6 to 8.0)
	B	do ..	7.1 (2.4 to 12.0)	4.9 (1.6 to 9.6)
	C	do ..	6.9 (2.4 to 12.0)	3.0 (1.6 to 8.0)
	Average		7.1 (2.4 to 12.0)	4.2 (1.6 to 9.6)
1144	Winesap:			
	A	Oct. 23	5.8 (2.4 to 8.0)	No starch present.
	B	do ..	5.9 (2.4 to 9.6)	No starch present.
	Average		5.8 (2.4 to 9.6)	
1156	Winesap:			
	A	Nov. 8	7.2 (1.7 to 15.8)	6.4 (2.6 to 11.4)
	B	do ..	5.5 (0.8 to 11.4)	7.0 (3.5 to 11.4)
	Average		6.3 (0.8 to 15.8)	6.7 (2.6 to 11.4)
1165	Winesap	Nov. 18	No starch present.	No starch present.

COLD-STORAGE APPLES.

1142	Winesap:			
	A	Oct. 23	5.9 (3.0 to 12.0)	No starch present.
	B	do ..	6.0 (2.4 to 11.0)	No starch present.
	Average		5.9 (2.4 to 12.0)	No starch present.
1143	Winesap:			
	A	Oct. 23	5.6 (1.6 to 9.6)	No starch present.
	B	do ..	5.7 (2.4 to 11.0)	No starch present.
	Average		5.6 (1.6 to 11.0)	
1147	Ben Davis: ^b			
	A	Oct. 23	4.3 (1.7 to 8.0)	No starch present.
	B	do ..	3.8 (1.7 to 7.0)	No starch present.
	C	do ..	4.9 (1.7 to 10.0)	No starch present.
	D	do ..	5.0 (1.7 to 8.8)	No starch present.
	Average		4.5 (1.7 to 10.0)	No starch present.
1148	Ben Davis: ^a			
	A	Oct. 23	6.3 (1.7 to 10.5)	No starch present.
	B	do ..	4.8 (1.7 to 9.6)	No starch present.
	C	do ..	5.3 (1.7 to 10.5)	No starch present.
	D	do ..	6.0 (2.6 to 8.8)	No starch present.
	Average	do ..	5.6 (1.7 to 10.5)	No starch present.

^a Picked September 15.^b Picked August 15.

WEAKENING OF CELL WALLS AND INCREASE OF INTERCELLULAR AIR.

During the fall of 1901, in the course of the examination of certain varieties, the intercellular air in the flesh of the fruit seemed to increase constantly. This led to the making of some determinations of the specific gravity of apples at different periods. The only tests, however, which were made during the first season's work, were carried out in December. The next summer and autumn a number of estimations were made at different stages in the growth and maturity of the fruit, the results showing that the specific gravity diminishes to the extent of 2 to 5 per cent. The results given, however, are the average specific gravities of from two to four specimens, and toward the latter part of the experiment it became apparent that the results would have possessed more value if a larger number of specimens had been used. In determining this factor the entire apple was used, and hence is not to be considered as representing the exact specific gravity of the flesh, though comparisons made for the purpose of this work showed that the results were only slightly below that of the flesh when taken alone. The results of this work are tabulated in Table X.

TABLE X.—*Specific gravity of whole fruit.*

Serial number.	Variety.	Date of analysis.	Specific gravity.
23667	Baldwin.....	Dec. 10, 1901	0.823
23670	Jonathan.....	do.....	.799
25221	York Imperial.....	July 22, 1902	.938
25219	Grimes.....	do.....	.8506
23669	Northern Spy.....	Dec. 10, 1901	.802
23668	Rhode Island Greening.....	do.....	.823
23658	Ben Davis.....	do.....	.753
25218	do.....	July 22, 1902	.823
1126	Winesap.....	Oct. 7, 1902	.8529
1165	do.....	Nov. 18, 1902	.8284
a 2149	do.....	Jan. 20, 1903	.8726
b 2151	do.....	do.....	.8480
1120	Rhode Island Greening.....	Sept. 15, 1902	.8337
1127	do.....	Oct. 7, 1902	.8297
1177	do.....	Nov. 18, 1902	.8272
2021	do.....	Jan. 15, 1903	.8083
2618	do.....	Jan. 28, 1903	.7994
1121	Northern Spy.....	Sept. 15, 1902	.8513
1128	do.....	Oct. 7, 1902	.8275
1178	do.....	Nov. 18, 1902	.8092
c 1146	Ben Davis.....	Oct. 23, 1902	.7538
1161	do.....	Nov. 18, 1902	.7832
1176	do.....	do.....	.7890
a 1147	do.....	Oct. 23, 1902	.7754
2148	do.....	Jan. 20, 1903	.7722
b 1148	do.....	Oct. 23, 1902	.7919
2150	do.....	Jan. 20, 1903	.7961
1175	do.....	Nov. 18, 1902	.7799
2612	do.....	Jan. 28, 1903	.7698
2887	do.....	Feb. 19, 1903	.7720
3582	do.....	Mar. 3, 1903	.7680
d 2803	do.....	Mar. 18, 1903	.7531
d 1494	do.....	Mar. 30, 1903	.7736
d 1671	do.....	Apr. 15, 1903	.7684
d 5915	do.....	Apr. 29, 1903	.7685
d 6078	do.....	May 12, 1903	.7732
2613	do.....	Jan. 28, 1903	.7507
3583	do.....	Mar. 3, 1903	.7558
e 4520	do.....	Mar. 31, 1903	.7645
e 5914	do.....	Apr. 29, 1903	.7703
e 6250	do.....	May 26, 1903	.7529

a Picked August 15.

b Picked September 15.

c Picked September 15-20.

d Used in respiration experiment, cellar temperature.

e Used in respiration experiment, cold-storage temperature.

The appearance of more intercellular air in the tissues and the consequent lessening of the specific gravity of the fruit are also correlated with the "mealiness" of the fruit. It is a matter of common knowledge that certain apples when ripe are more "mealy" than others, and that in some varieties this quality increases greatly just before decay. If a small piece of a mealy apple is carefully crushed and then examined under the microscope, it is found that the cells, for the most part, have not been ruptured, but have simply been separated from each other. This has been made possible by the softening of the middle lamellæ of the cell walls, which occurs in the last stages of ripening. The middle lamella becomes so very weak that under a slight shearing force it gives way, thus allowing the adjacent cells to separate without rupturing their walls.

In the earlier stages of the ripening of the fruit a very different condition exists, because then the middle lamellæ are strong, and the cells rupture instead of splitting apart when subjected to pressure. The softening of the middle lamella may also be artificially accomplished by boiling. A striking illustration of this is found in the process of making apple butter, in which boiling is continued until the cells fall apart when stirred. The readiness with which this occurs varies with the different varieties and the degree of ripeness attained. This difference in the behavior of the middle lamella in the readiness with which it is dissolved by heat the housewife recognizes under the common expression of difference of "cooking qualities," and she selects apples with regard to this property as well as that of flavor. The softening of the middle lamella, whatever its cause, serves, in part at least, to explain the difference in the mealy texture of apples which may have the same percentage of juice, since in one case the process of mastication does not accomplish the rupturing of the cells, which, like little bags or capsules, retain the juice. Freezing also appears to have a similar softening effect upon the lamella, inasmuch as apples which have been frozen have a decidedly mealy texture and soft consistency.

In normal ripening the softening of the middle lamella results in a slight change in the contour of the cell wall, so that the cell is more regular in outline. The intercellular space is increased, and, as there is usually but little liquid to fill the cavity, the amount of intercellular air is increased. When viewed under the microscope, the presence of air in some varieties is very noticeable indeed when nearly ripe, while in the greener condition it is but little in evidence.

An experiment was conducted to show the difference in the readiness of disintegration (dependent on the softening of the middle lamella in the same fruits) under different conditions of storage. Cubes of as nearly the same size as possible were cut from two samples and placed in test tubes with a given amount of water. One set

of samples used was taken from Ben Davis apples (No. 4494) which had been kept at cellar temperature and contained 14.7 per cent of solids. The other set was of the same variety, but had been kept in cold storage and contained 14.3 per cent of solids. These samples were placed under as nearly similar conditions as regards containers and amount of water as possible and were shaken alike for four minutes. The solids of the nondisintegrated portions were then determined. While shaking the specimens they took up more water, so that the solids were reduced to about 12 per cent. This made it necessary to make the calculations on the basis of the solids present, and not on the weight as found before and after shaking. The results showed that of those specimens taken from cellar storage over 50 per cent disintegrated in the shaking process, while with the others less than 20 per cent of loss occurred. It seemed probable that the former apples would be more mealy to the taste than the latter, even though they contained an equal amount of juice, and this was found to be the case.

MACROSCOPIC STUDIES.

TERMINOLOGY.

The difficulty experienced in recording the changes occurring during the disappearance of starch led to the free use of photography to record these results. The cut sections were exposed for a few moments to fumes of sulphur dioxid, rinsed in clear water, and then dipped for about 10 minutes in a deep straw-colored solution of iodine in potassium iodid solution. The starchy regions were of course strongly differentiated by this treatment, and from the sections thus prepared the making of suitable photographs was easily accomplished.

Before taking up the discussion of the macroscopic features of the occurrence of starch it is necessary to locate in the fruit certain zones which are of more or less importance in understanding the changes which occur in the ripening. It is not our purpose, however, to go into a discussion of the morphological features of the apple which do not seem to be of vital importance in this connection. For the more perfect understanding of this part of our work it has seemed necessary to devise a few new names, since in botanical literature no terms have been found which exactly designate certain parts of the tissue considered.

If an apple is cut in half, along the equator half way between the stem and the calyx "eye," and the cut ends are examined, certain features concerning the fruit are presented very clearly, as shown in figure 30. The most prominent among these are the oval or elliptical shaped seed cells with the sharp points clustered at the center. The number of these cells is usually five, though specimens having either four or six are not infrequently found.

Some distance out in the fleshy portion is a line more or less distinctly shown, and commonly of a greenish color, called the core line. This line is divisible into the same number of sections as the seed cells, the ends of each section bending in somewhat toward the seed cells. The points where two of these portions, usually considered the carpels,^a meet may for convenience be called the "carpel seams." Midway between the carpel seams and on the core line are greenish dots—the cut ends of fibrovascular bundles which might be called "carpel ribs," since they are the midribs of the carpel leaves. Extending down between the seed cells are wedge-shaped portions of flesh, which may be called "core wedges."

Outside the core line in the torus flesh are other zones which figure in the course of ripening, though probably they are of little other interest. Their prominence varies in different varieties, though there is a general similarity for all the varieties so far examined. The most important of these are oval-shaped regions, as seen in cross section, situated in the midst of the torus flesh, and having the small ends in juxtaposition to the fibrovascular bundles.

Flanking the sides of each oval region, and extending toward the fibrovascular bundles, are two fan-shaped portions which meet at the carpel seams and ribs, and form what might be termed "V-zones." With these various zones in mind the photographs illustrating the various stages in the ripening of apples may be clearly described.

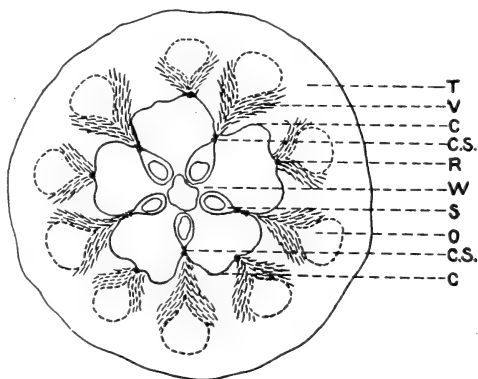


FIG. 30.—Diagrammatic cross section of apple showing location of various regions and structures: T, torus flesh; C, core line; S, seed cells; O, oval regions; C.S., core seam; W, core wedge; R, carpel rib; V, V-zone.

CHANGES IN STARCH CONTENT.

The changes in starch content, and its location, for 4 varieties of apples at various stages of development are shown in Plates II to V, 2 other varieties, not illustrated, being discussed from this point of view.

^aTransactions of Ill. Horticultural Society, 1894, new series, 28: 125, Possibilities of Improvement, T. J. Burrill.

WINTER PARADISE (PLATE II).

The samples of this variety examined August 17 indicated by the amount of starch present that ripening had already begun. The starch had nearly all gone from the core wedges, but the torus flesh was heavily charged with it. On September 24 the starch had practically disappeared from within the core line, and there was a marked diminution in that of the torus flesh, especially in the oval zones. On October 15 most of the starch had disappeared, though some still persisted in the region of the V-zones and fibrovascular bundles of the outer portion. On October 23 only a trace of starch could be found, except in bruised spots, and by the end of the month it had all disappeared.

From the starch content it would appear that the ripening period of the Winter Paradise begins about August 15, and is ended by the last week of October, unless kept under special conditions of storage. Since the ripening of the core region is practically completed before the changes in the starchy portions of the torus flesh begin, the ripening may be divided into two fairly distinct steps. In the Winter Paradise the first period of ripening extended from about August 15 to about the middle of September, and the second period from that time to the last part of October.

BEN DAVIS (PLATE III).

On August 17 the fruit presented an even dense deposit of starch throughout all parts of the flesh. On September 24 the starch had begun to decrease. It was most pronounced in the core wedges, and a very slight decrease in the density of color produced with iodine was noticeable in certain parts of the torus flesh. When examined again, on October 23, a decided change was found to have occurred during the preceding month, and only small amounts of starch remained in the V-zones and the periphery, and a week later, on October 30, no starch at all remained.

In the Ben Davis the ripening season is rather short, setting in about the middle of September and being completed about the last of October.

HUNTSMAN (PLATE IV).

The first fruit in this series was examined August 17, at which time the ripening apparently had not begun, unless perhaps very slightly, as there seemed to be small amounts of starch in the inner portions of the "core wedges." However, it is usually true that there is less starch in the core wedges than elsewhere, even in the green fruit.

On September 24 the iodine test showed the starch to be nearly all gone from the core region. Traces still remained in the tissues flanking the carpel seams within the core flesh. A decrease was also

apparent in the outer portions, producing a slightly streaked appearance. The beginning of the decrease in the amount of starch in the oval zones is not so plain in this as in some other varieties.

On October 15 the starch was all gone and the fruit had every appearance of being fully ripe. Specimens were also examined on October 23, October 30, and November 5, but no material change was noticeable except an increasing mealiness in the texture of the tissue.

From these facts it would appear that the ripening of the Huntsman begins about the last of August. The first period extends over about three or four weeks and the second stage is completed by the middle of October.

YELLOW TRANSPARENT (PLATE V).

On July 24 this variety was found to have reached the second stage in the course of ripening. It is true, however, that considerable starch still remained within the core line while a decided loss of starch seemed to be occurring in the torus flesh. The specimens examined on July 29 showed but little change from this condition, and nearly a month elapsed before specimens were again examined. The photographs made showed how slowly the second stage of ripening sometimes progresses. At this time it was found that the starch was all gone from within the core line, while a considerable amount still remained in the torus flesh. Specimens examined again August 31 showed that the starch had completely disappeared.

It seems that the ripening period for the Yellow Transparent is approximately from the middle of June to the latter part of August. This variety also illustrates how irregularly many of the summer varieties mature, many specimens being much in advance of others on the same tree as regards ripeness. It is also true that this variety does not strictly follow a given order of ripening, as is the case with most winter varieties.

EARLY STRAWBERRY (NOT ILLUSTRATED).

These specimens had apparently ripened considerably while en route to the laboratory. In this variety the starch had very largely disappeared from within the core line by June 25. Some streaking was also appearing in the torus flesh. On July 24 the starch had decreased very much in the outer portion. The V-zones were very well marked in this variety. The specimens of July 29 showed but little advance over those of the 24th, except that there seemed to be a slight weakening in the intensity of the iodine reaction. Unfortunately this set is incomplete both at the beginning and the ending of the maturing period.

BOUGH (NOT ILLUSTRATED).

The apples of this variety ripen so rapidly that the time necessary to bring them from the orchards gave considerable opportunity for marked changes to occur before the specimens reached the laboratory.

The apples used were obtained from two orchards, one in Delaware and the other at Geneva, N. Y. In both cases the samples had reached an advanced stage of ripening before they arrived at the laboratory. Those arriving from Geneva, August 24, showed the starch to be nearly gone from within the core line—only a little remaining near the carpel seams. Outside this line a slight tendency to streak was becoming evident.

In the specimens arriving one week later, on August 31, the starch within the core limit had entirely disappeared. Outside the core line strong streaking had occurred; at each of the V-zones were regions which stained very black with iodine. On September 8 the starch had very much decreased, the greater part appearing in the V-zones.

Of the several varieties observed the Bough is one of the most striking illustrations of the changes in the quantity of starch in the various zones of the tissues, though the location of the oval zone perhaps is not so well defined as in some others.

CONCLUSIONS.

The macroscopic studies on the localization of starch in the process of ripening led to the following conclusions:

The ripening takes place in two more or less well-defined stages. The first of these occurs within the core line, the second in the torus flesh. The first ordinarily begins by a decrease in the starch content in the tips of the core wedges. This decrease extends outward to the core line, and the last regions within this zone to lose their starch are the fan-shaped portions flanking the carpel seams of the core region.

The second stage ordinarily sets in somewhat before the first is fully completed, and is usually heralded by starch-free streaks appearing in the midst of the torus flesh. Though not at all confined to them, the first indication of the disappearance of the starch is commonly most pronounced in the oval zones. These zones increase in size and other radial streaks appear in the fleshy portion until in the middle part of the torus flesh there exists but little starch. Usually, extending outward from the fibrovascular bundles of the carpel ribs and seams, there are fan-shaped (or V-shaped) portions which may best be termed V-zones, and which, together with the peripheral 1.5 to 3 mm of the flesh, are among the last to lose their starch.

Another fact shown is one well recognized by horticulturists, namely, that summer apples ripen much more irregularly than the

winter varieties. Hence, although a longer time is necessary to complete the work, for the purpose of studying the chemical changes occurring in apples it would seem that the winter varieties are to be preferred. It must be borne in mind, however, that much of the ripening in these varieties occurs after they are picked, while the summer varieties are more nearly ripened before picking time.

It is also demonstrated that although there is a general similarity between different varieties in their ripening, there are also well-defined differences.

DESCRIPTION OF PLATES.

[The serial numbers refer to the analyses of the same specimens which appear in the tables in the earlier part of the bulletin.]

PLATE I.—*Photomicrographs of apple starch.*

Fig. 1. Apple tissue, showing starch in apple cells ($\times 40$).

Fig. 2. Apple starch ($\times 200$).

* PLATE II.—*Winter Paradise.*

	Per cent.
Fig. 1. Serial No. 6925; August 18, 1903; starch content.....	1.69
Fig. 2. Serial No. 7260; September 25, 1903; starch content.....	1.19
Fig. 3. Serial No. 7287; October 15, 1903; starch content.....	.61
Fig. 4. Serial No. 7296; October 23, 1903; starch content.....	.50
Fig. 5. Serial No. 7305; October 30, 1903; starch content.....	.28
Fig. 6. Serial No. 7310; November 5, 1903; starch content.....	None.

PLATE III.—*Ben Davis.*

Fig. 1. Serial No. 6923; August 18, 1903; starch content.....	3.16
Fig. 2. Serial No. 7258; September 25, 1903; starch content.....	2.40
Fig. 3. Serial No. 7294; October 23, 1903; starch content.....	.94
Fig. 4. Serial No. 7303; October 30, 1903; starch content.....	.38
Fig. 5. Serial No. 7308; November 5, 1903; starch content.....	None.

PLATE IV.—*Huntsman.*

Fig. 1. Serial No. 6924; August 18, 1903; starch content.....	2.69
Fig. 2. Serial No. 7259; September 25, 1903; starch content.....	1.00
Fig. 3. Serial No. 7286; October 15, 1903; starch content.....	.30
Fig. 4. Serial No. 7295; October 23, 1903; starch content.....	.72
Fig. 5. Serial No. 7304; October 30, 1903; starch content.....	.19
Fig. 6. Serial No. 7310; November 5, 1903; starch content.....	None.

PLATE V.—*Yellow Transparent.*

Fig. 1. Serial No. 6890; July 24, 1903; starch content.....	0.35
Fig. 2. Serial No. 6894; July 29, 1903; starch content.....	.53
Figs. 3 and 4. Serial No. 7054; August 24, 1903; starch content.....	None.
Fig. 5. Serial No. 7083; August 31, 1903; starch content.....	None.

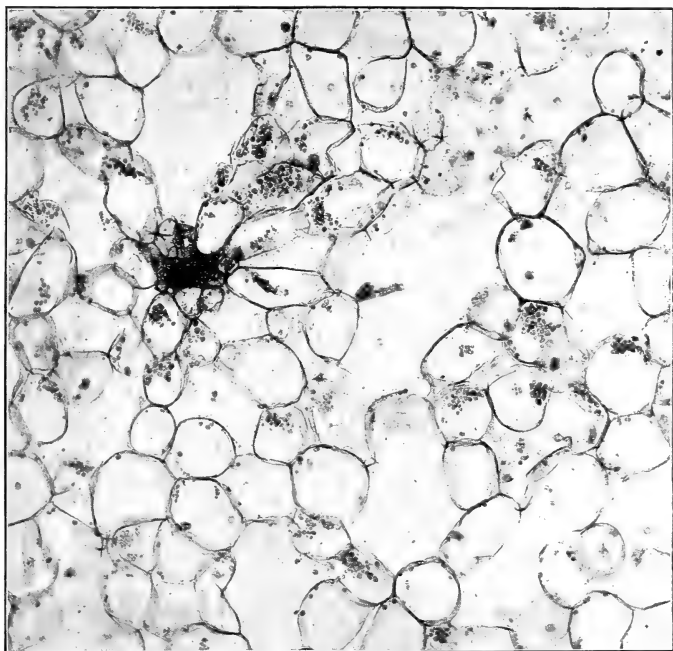


FIG. 1.—APPLE TISSUE, SHOWING STARCH IN CELLS. (ENLARGED 40 DIAMETERS.)

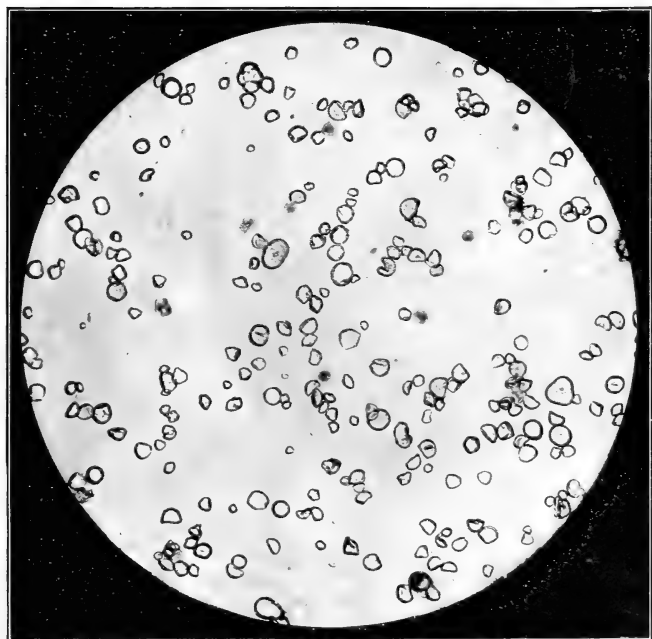


FIG. 2.—APPLE STARCH. (ENLARGED 200 DIAMETERS.)

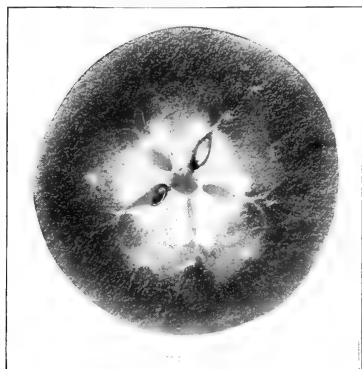


Fig. 1.—August 18, 1903.

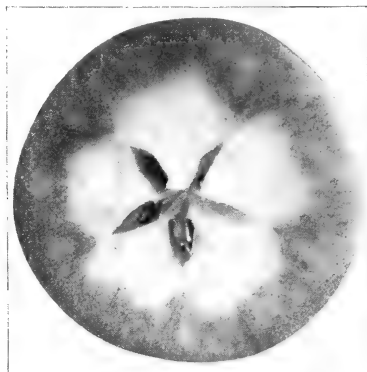


Fig. 2.—September 25, 1903.

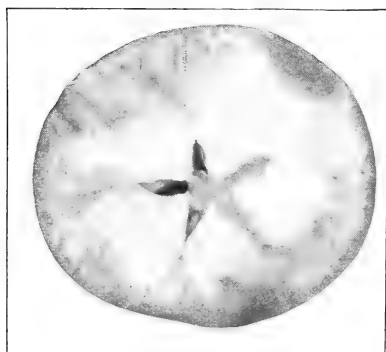


Fig. 3.—October 15, 1903.

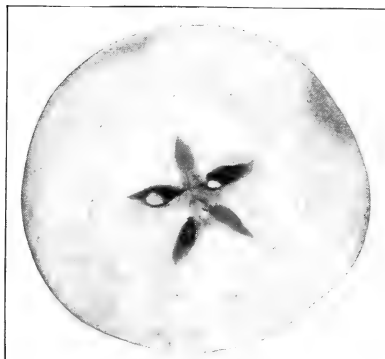


Fig. 4.—October 23, 1903.



Fig. 5.—October 30, 1903.

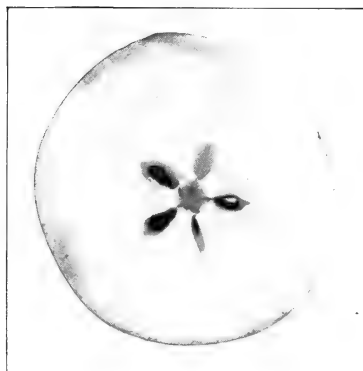


Fig. 6.—November 5, 1903.

LOCATION AND GRADUAL DISAPPEARANCE OF APPLE STARCH, WINTER PARADISE.

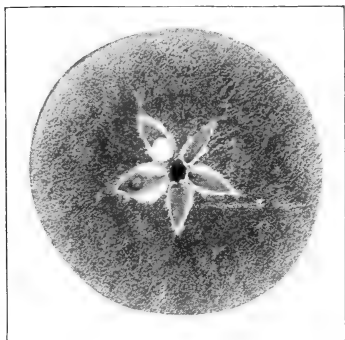


Fig. 1.—August 18, 1903.

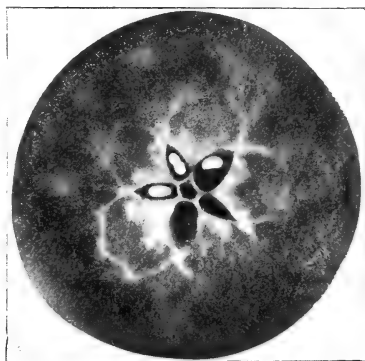


Fig. 2.—September 25, 1903.

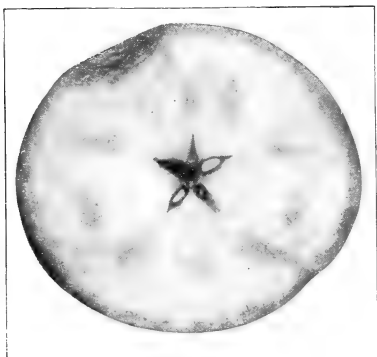


Fig. 3.—October 23, 1903.

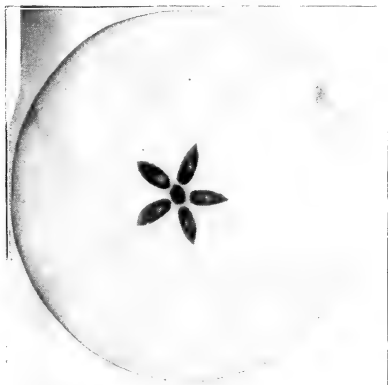


Fig. 4.—October 30, 1903.

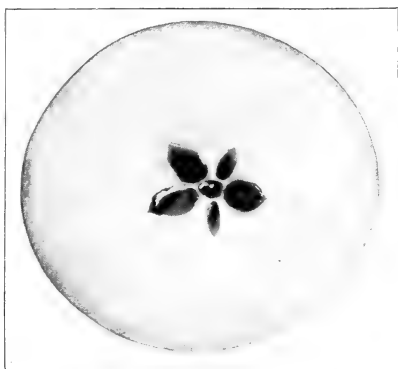


Fig. 5.—November 5, 1903.

LOCATION AND GRADUAL DISAPPEARANCE OF APPLE STARCH, BEN DAVIS.

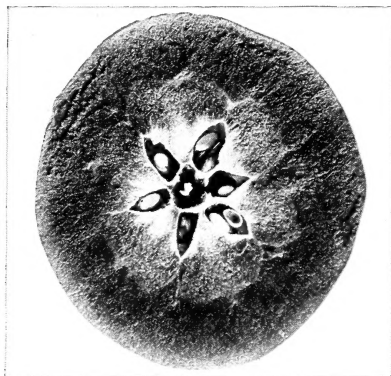


Fig. 1.—August 18, 1903.

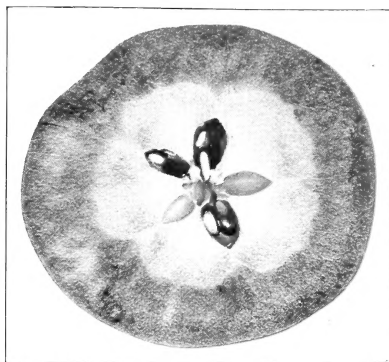


Fig. 2.—September 25, 1903.

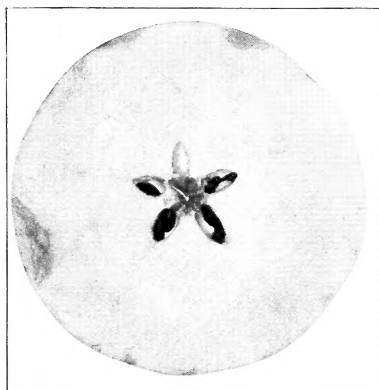


Fig. 3.—October 15, 1903.

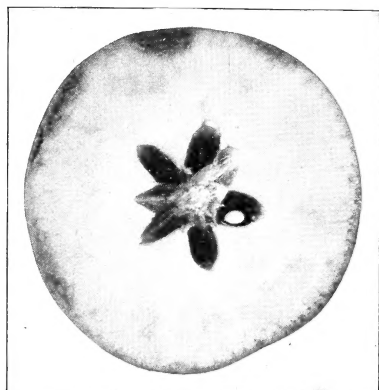


Fig. 4.—October 23, 1903.

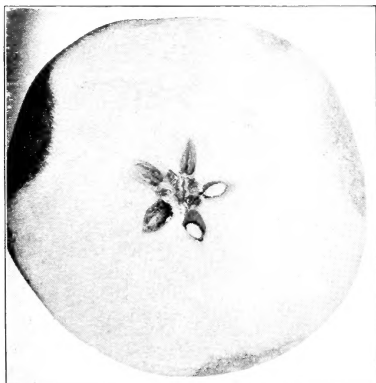


Fig. 5.—October 30, 1903.

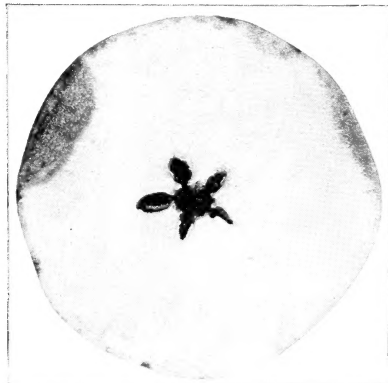


Fig. 6.—November 5, 1903.

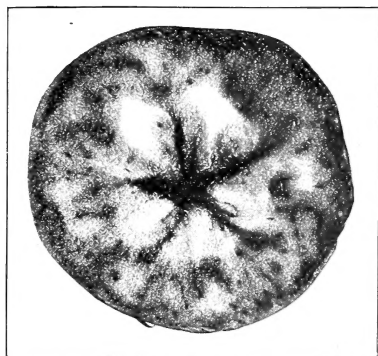


Fig. 1.—July 24, 1903.

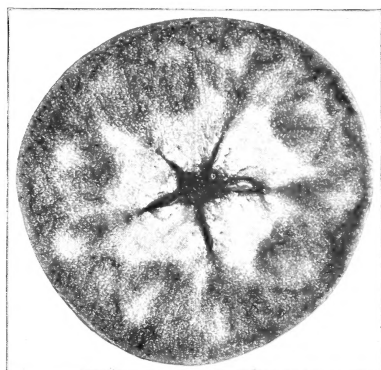


Fig. 2.—July 29, 1903.

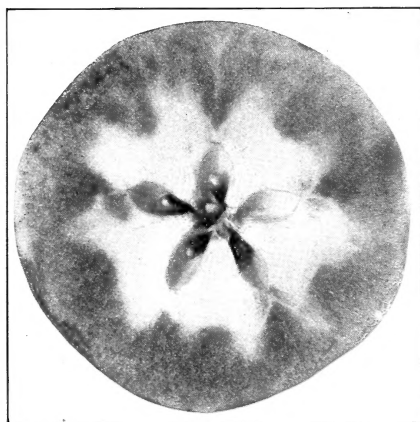


Fig. 3.—August 24, 1903.

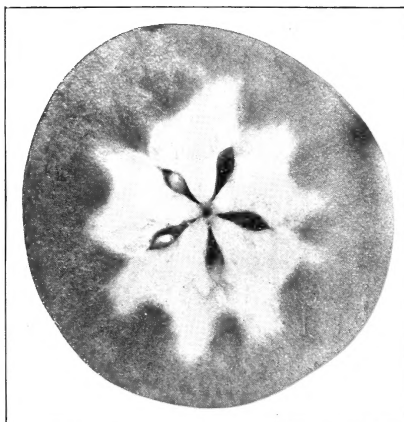


Fig. 4.—August 24, 1903.

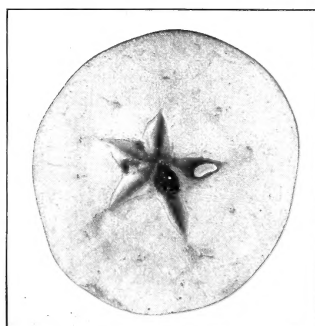


Fig. 5.—August 31, 1903.

LOCATION AND GRADUAL DISAPPEARANCE OF APPLE STARCH, YELLOW TRANSPARENT.

